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(54) Title: MEFLOQUINE, NELFINAVIR AND SAQUINAVIR AS NOVEL AGENTS FOR NEURODEGENERATIVE AND (NEURO-) INFLAMMATORY DISEASES

(57) Abstract: The present invention generally relates to the neuroprotective, anti-apoptotic and anti-inflammatory activity of mefloquine, nelfinavir and saquinavir and derivatives thereof based on recently discovered interactions with prohibitin and estrogen receptors and the inhibition of mitochondrial voltage- dependent anion channel 1. Thus, mefloquine, nelfinavir and saquinavir and derivatives thereof may be used as medicaments for the prevention and/or treatment of neurodegenerative and inflammatory, particularly neuroinflammatory diseases.

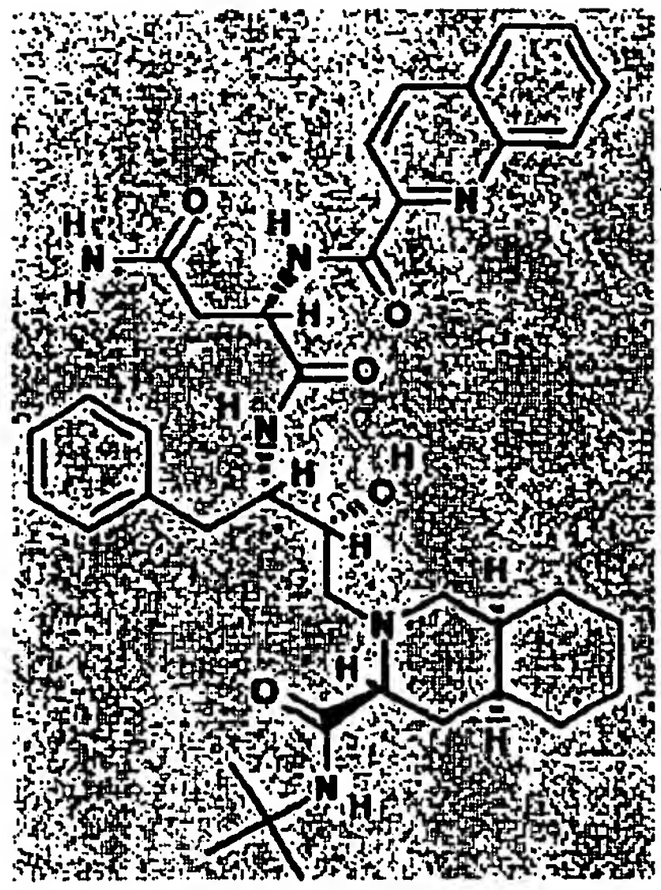
Mefloquine, Nelfinavir and Saquinavir as novel agents for neurodegenerative and (neuro-) inflammatory diseases

Description

The present invention generally relates to the neuroprotective, anti-apoptotic and anti-inflammatory activity of mefloquine, nelfinavir and saquinavir and derivatives thereof based on recently discovered interactions with prohibitin and estrogen receptors and the inhibition of mitochondrial voltage-dependent anion channel 1. Thus, mefloquine, nelfinavir and saquinavir and derivatives thereof may be used as medicaments for the prevention and/or treatment of neurodegenerative and inflammatory, particularly neuroinflammatory diseases.

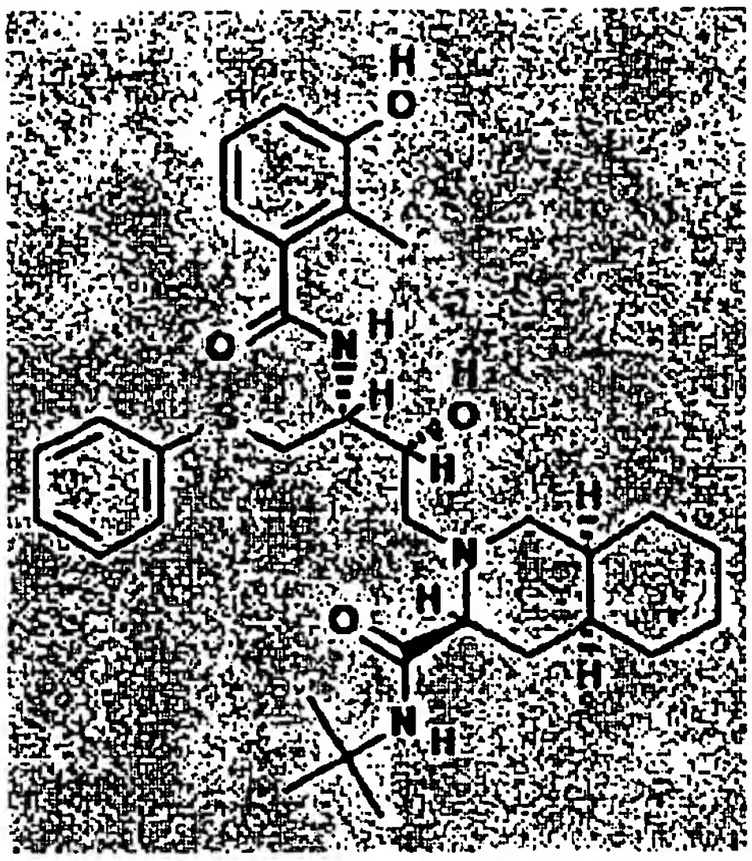
Nelfinavir (Viracept®) and saquinavir (Invirase® or Fortovase®) are known as anti-HIV drugs called HIV protease inhibitors. Further HIV protease inhibitors of the same class of compounds are amprenavir (Agenerase®), indinavir (Crixivan®), lopinavir (Kaletra®), ritonavir (Norvir®) and atazanavir (Reyataz®). These compounds are used for treatment of HIV.

Nelfinavir is usually administered in the form of (3S-(2(2S*,3S*), 3α,4aβ,8aβ))-N-(1,1-dimethylethyl)decahydro-2-(2-hydroxy-3-((3-hydroxy-2-methylbenzoyl)amino)-4-(phenylthio)butyl)-3-isoquinolinecarboxamide; The compound, its manufacture, its mechanism of action and its clinical efficacy have been described in the state of the art.



Nelfinavir

Saquinavir is usually administered in the form of Saquinavir mesylate (S)-N-((αS)-α-((1R)-2-((3S,4aS,8aS)-3-(tert-butylcarbamoyl)octahydro-2(1H)-isoquinolyl)-1-hydroxyethyl) phenethyl)-2-quinaldamidosuccinamide monomethanesulfonate (salt); DRG-0164; Fortovase®; Invirase®; Ro 31-8959/003; Saquinavir monomethanesulfonate salt; butanediamide, N(sup 1)-(3-(3-(((1,1-dimethylethyl)amino) carbonyl)octahydro-2(1H)-isoquinolinyl)-2-hydroxy-1- (phenylmethyl)propyl)-2-((2-quinolinylcarbonyl)amino)-, (3S-(2 (1R*(R*),2S*),3α,4aβ,8aβ))-, monomethanesulfonate (salt); N-[1-benzyl-2-hydroxy-3-[[3-(tert-butylcarbamoyl)-1,2,3,4,4a,5, 6,7,8,8a-decahydroisoquinol-2-yl]]propyl]-2-(2- quinolylcarbonylamino)succinamide; methanesulfonic acid. The compound, its manufacture, its mechanism of action and its clinical efficacy have been described in the state of the art.



Saquinavir

The assumed antiapoptotic properties of this class of compounds have already been disclosed (Badley A. D. In vitro and in vivo effects of HIV protease inhibitors on apoptosis. Cell Death Differ. (2005) Mar 11; [Epub ahead of print]; Phenix B. N., Lum J.J., Nie Z., Sanchez-Dardon J., and Badley A. D., Antiapoptotic mechanism of HIV protease inhibitors: preventing mitochondrial transmembrane potential loss; Blood. 98, 1078-1085 (2001)).

Mefloquine (Lariam®) is known as an anti-malaria drug which has high efficacy in treating the widespread chloroquine-resistant *Plasmodium falciparum* strains. Mefloquine is used both for prophylaxis and treatment of malaria and is relatively well tolerated.

Mefloquine is usually administered in the form of mefloquine hydrochloride *(R*,S*)-(+-)-α-2- piperidinyl-2,8-bis (trifluoromethyl)-4-quinolinemethanol-monohydrochloride;* DL-erythro-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol monohydrochloride; WR-142490; Ro-21-5998; Lariam®. $C_{17}H_{17}ClF_6N_20$; mol wt 414.78. C 49.23%, H 4.13%, Cl 8.55%, F 27.48%, N 6.75%, O 3.86%. The compound, its manufacture, its mechanism of action and its clinical efficacy have been described extensively in C. J. Ohnmacht

et al. *J. Med. Chem.* 14, 926 (1971); DE patent 28 06 909, *CA.* 90, 22838q (1979); U.S. patent 4,507,482; J. T. Blackwell, *J. Med. Chem.* 17, 210 (1974); M. W. Davidson *et al, J. Med. Chem.* 20, 1117 (1977); R. E. Brown *et al., Life Sci.* 25, 1857 (1979); Photochemistry: G. A. Epling, U. C. Yoon, *Chem. Letters* 1982 (2), 211; Pharmacokinetics: D. E. Schwartz *et al., Chemotherapy (Basel)* 28, 70 (1982). HPLC determination: 1. M. Kapetanovic *et al, .1. Chromatog.* 277, 209 (1983); G. M. Trenholme *et al., Science* 190, 792 (1975); F. Tin *et al. Bull. WHO* 60, 913 (1982); J. M. Kofe Ekue *et al., ibid.* 61, 713 (1983); J.-M. de Souza *ibid.* 809, 815 and P. Lim in *Analytical Profiles of Drug Substances* vol. 14, K. Florey, Ed. (Academic Press, New York, 1985) pp 157-180 (all citations from Merck Index, 12th edition, 1996).

The relatively new antimalarial mefloquine (Lariam®) has become extremely popular due to its efficacy in treating the wide-spread chloroquine-resistant *Plasmodium falciparum* strains. Mefloquine is known both for severe neurologic and psychiatric adverse effects associated with its use.

There have also been reports about neurotoxic effects of mefloquine at higher dosages (Rendi-Wagner P. et al., Acta Trop. 81, 167-173 (2002). The mechanisms of these adverse effects are discussed controversially in the literature. Toxic encephalopathy appears to be one of the serious neurological manifestations which is slowly reversible depending on individual predisposition. Self-administration schemes can be therefore both most useful and dangerous due to expected benefits and potential risks. (Nicolas X. et al., Presse Med. 30, 1349-1350 (2001); Dow G. S. et al., Antimicrob. Agents Chemother. 48, 2624-2632 (2004). There are indications that disruption of neuronal calcium homeostasis can induce an ER stress response at physiologically relevant concentrations, thus contributing to the neurotoxicity of the drug in vitro (Dow G. S. et al., Malar. J. 2, 14 (2003). There are other indications, that mefloquine shows stereoselective brain uptake in humans and rats and is a substrate and an inhibitor of the efflux protein P-glycoprotein (Barraud de Lagerie S. et al., Br. J. Pharmacol. 141,

1214-1222 (2004)). Or, that mefloquine inhibits certain gap junction channels at concentrations between 1 and 100 µM (Cruikshank S. J. et al., Proc. Natl. Acad. Sci. USA 101, 12364-12369 (2004)). Also, it has been reported that the (-)-(R,S)-enantiomer of mefloquine is a potent adenosine A2A receptor antagonist (Weiss et al. ... (2003)). Moreover, other reports show inhibition of volume-regulated and calcium-activated chloride channels by mefloquine (Maertens et al., J. Pharmacol. Exp. Ther. 295, 29-36 (2000)).

The crucial molecular features correlated with neurotoxicity are under investigation and certain molecular features defining neurotoxicity are emerging. There is an important hydrogen bond acceptor (lipid) function, an aliphatic hydrophobic function, and a ring aromatic function specifically distributed in the 3D surface of the molecule. Mapping of the 3D structures of a series of structurally diverse quinolines to the pharmacophore allowed accurate qualitative predictions of neurotoxicity (or not) to be made (Dow G. S. et al., supra (2004)).

In contrast to reports on neurotoxicity other reports cite mild side effects (Wattanakoon Y. et al., Southeast Asia J. Trop. Med. Public Health 34, 542-545 (2003)) or even recommend use of mefloquine during pregnancy (Adam I. et al., Saudi Med. J. 25, 1400-1402 (2004) or in infants (Dubos F. et al., Pediatr. Infect. Dis. J. 23, 679-618 (2004)). Interestingly, there is a recent theoretical study coming to the conclusion that mefloquine, along with other approved heterocyclics may have neuroprotective properties, potentially acting on a mitochondrial target (Stavrovskaya I. G., et al. J. Exp. Med. 200, 214-222 (2004)). Moreover, a neuroprotective activity of mefloquine based on its activity as a purinergic receptor antagonist is suggested by Fletcher et al. (US Pat. 6,197,788), (-)-mefloquine is suggested to block purinergic receptors and is claimed for the treatment of movement or neurodegenerative disorders.

Taken together there are many suggestions of potential new targets of mefloquine and some controversy about the severity, nature and mechanism

of the neurotoxic side effects. There are thus no proven findings at present regarding the sphere of medical application for mefloquine.

In the present application novel modes of action and novel targets for mefloquine, nelfinavir and saquinavir and derivatives thereof are described, thus establishing novel medical applications. These novel targets and their implication for medical applications, particularly in the field of neuroprotection, are described below.

The voltage-dependent anion selective channel-1 (VDA1) is a constitutive mitochondrial protein which can induce pro-apoptotic mitochondrial membrane permeabilisation and thus a potential target for the design and development of therapeutic strategies.

Since its introduction to clinical use in 1985, there have been numerous reports of severe adverse neurological and psychiatric effects including acute psychosis, affective disorders, acute confusional states and seizures (Le Bras M. et al., Histol. Histopathol. 20, 205-219 (2005)).

It has been shown that the voltage dependent anion selective channel (VDAC) in the outer membrane is a specific benzodiazepine binding site in mitochondrial membranes (Slocinska M. et al., Acta Biochim. Po. 54, 953-962 (2004)). VDAC proteins have also been implicated in the pathology of models for amytrophic lateral sclerosis (ALS), a fatal neurodegenerative disease characterized by progressive motor neuron death (Fukada K. et al., Mol. Cell Proteomics 12, 1211-1223 (2004)). Moreover it has been shown that VDAC proteins are tyrosine-phosphorylated under hypoxic conditions suggesting that tyrosine phosphorylation may then contribute to the modulation of VDAC protein function/conformation or interaction with other proteins (Liberatori S. et al., Proteomics 4, 1335-1340 (2004)). Also a role as redox sensor has been suggested recently (Baker M. A. et al., Biofactors 21, 215-221 (2004)). This relates very well to the experimental examples described below.

In the context of potentially anti-apoptotic and cyto-/neuroprotective properties of mefloquine, nelfinavir and saquinavir described here it is noteworthy that certain hydrophobic hormones like thyroid hormone exert antiapoptotic effects via interaction with components of the mitochondrial transition pore (Yehuda-Shnaidman E. et al., Endocrinology, (2005)). This might relate to the identification of the repressor of estrogen receptor related activity (REA) as a further protein labelled by the reactive mefloquine, nelfinavir and saquinavir derivatives described in the examples below.

Prohibitins comprise a remarkably conserved protein family in eukaryotic cells with proposed functions in cell cycle progression, cancer, cellular stress management, senescence, apoptosis, and the regulation of mitochondrial activities (Liu H. et al., Proteomics 10, 3167-3176 (2004); Huang C. M. et al., Mass Spectrom. Rev. (2004); Wang K. J. et al., World of Gastroenterol. 10, 2179-2183, (2004); Wang S. et al. EMBO J. 23, 2293-2303 (2004); Gamble S. C. et al., Oncogene 23, 2996-3004 (2004); Joshi B. et al. Biochem. Biophys. Res. Commun. 312, 459-466 (2003); Fusaro G. et al., J. Biol. Chem. 278, 47853-47861 (2003)). Two prohibitin homologues, Phb1 and Phb2, assemble into a high molecular weight complex of approximately 1.2 MDa in the mitochondrial inner membrane, but a nuclear localization of Phb1 and Phb2 also has been reported (Tatsuta T. et al., Mol. Biol. Cell. 16, 248-259, (2005)). Prohibitin is also a tumor suppressor enriched in membrane microdomains, called lipid rafts and associated with viral infectious and inflammatory pathways, potentially via mitogen-activated protein kinase (e.g. Sharma A. et al., Proc. Natl. Acad. Sci. USA 101, 17492-17497 (2004)).

Recent reports list prohibitin among the secreted proteins of adipocytes (Wang P. et al., Cell. Mol. Life Sci. 61, 2405-2417, (2004)), moreover it has been established as a vascular marker of adipose tissue, which was target of a proapoptotic peptide as an antiobesity experimental treatment (Kolonin M. G. et al. Nat. Med. 10, 625-632 (2004)).

Recent data suggest that estrogen may exert neuroprotective effects against

β-amyloid-induced toxicity by activation of estrogen receptor-mediated pathways in cholinergic neurons as a model for the pathology of Alzheimer's disease. In addition, intracellular estrogen receptors are up-regulated by their cognate hormone even during exposure to neurotoxic agents, like β-amyloid1-40 (Marin R. et al., Neuroscience 121, 917-926 (2003)). Estrogen appears to be an important neuromodulatory molecule. REA (repressor of estrogen receptor activity) encodes a 37-kDa protein that is an ER-selective coregulator. Its competitive reversal of steroid receptor coactivator 1 enhancement of ER activity and its direct interaction with liganded ER suggest that it may play an important role in determining the sensitivity of estrogen target cells to antiestrogens and estrogens, especially in cancer (Montano M. M. et al., Proc. Natl. Acad. Sci. USA 96, 6947-6952, (1999); Simon S. L. et al., Cancer Res. 60, 2796-2799, (2000)). REA is recruited to the hormone-occupied ER, decreases the transcriptional activity of ER, both when ER is acting directly through DNA response elements as well as when it is tethered to other transcription factors. Administration of antisense REA resulted in a 2-4-fold increase in ER transactivation, implying that endogenous REA normally dampens the stimulatory response to estradiol (Delage-Mourroux R. et al., J. Biol. Chem. 275, 35848-35856, (2000)).

Estrogen antagonists are universally employed in the breast cancer therapy. The molecular mechanisms by which these agents inhibit cellular proliferation in breast cancer cells are not fully defined. Recent studies have shown the involvement of the E2F (family of transcription factors) pathway in tamoxifen-induced growth arrest. E2F repressor, like prohibitin and the chromatin modifiers Brg1/Brm are required for estrogen antagonist-mediated growth suppression through the estrogen receptor, and that their recruitment to native promoter-bound E2F is induced via a JNK1 pathway. Collectively, these findings suggest that the prohibitin/Brg1/Brm node is a major cellular target for estrogen antagonists, and thereby also implicate prohibitin/Brg1/Brm as potentially important targets for breast cancer therapy (Wang S. et al., supra, (2004)).

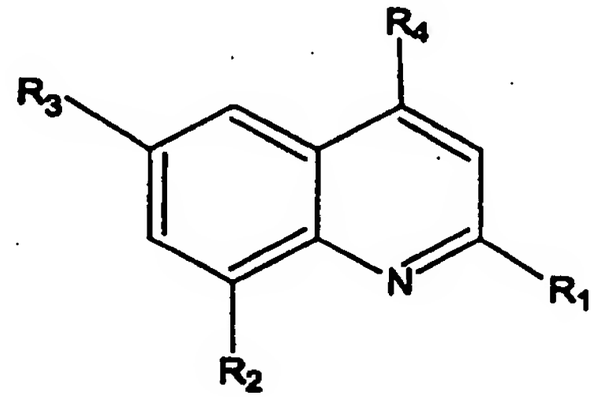
Of particular interest is the recently discovered interaction of REA with histone deacetylases, which have drawn interest as potential neuroprotective targets (Langley B. et al., Curr. Drug Targets CNS Neurol. Disord. 4, 41-50, (2005)). Acetylation and deacetylation of histone protein plays a critical role in regulating gene expression in a host of biological processes including cellular proliferation, development, and differentiation. Accordingly, aberrant acetylation and deacetylation resulting from the misregulation of histone acetyltransferases (HATs) and/or histone deacetylases (HDACs) has been linked to clinical disorders such as Rubinstein-Taybi syndrome, fragile X syndrome, leukemia, and various cancers. Aberrant HAT and HDAC activity may also be a common underlying mechanism contributing to neurodegeneration during acute and chronic neurological diseases, including stroke, Huntington's disease Amyotrophic Lateral Sclerosis and Alzheimer's disease (Langley B. et al. supra, (2005)).

Histone acetyltransferases and deacetylases are recruited by transcription factors and adapter proteins to regulate specific subsets of target genes. The repressor of estrogen receptor activity (REA) has been identified as a novel HDAC1-associated protein. REA also associates with the class II histone deacetylase HDAC5 (Kurtev V. et al., J. Biol. Chem 279, 24834-24843, (2004)).

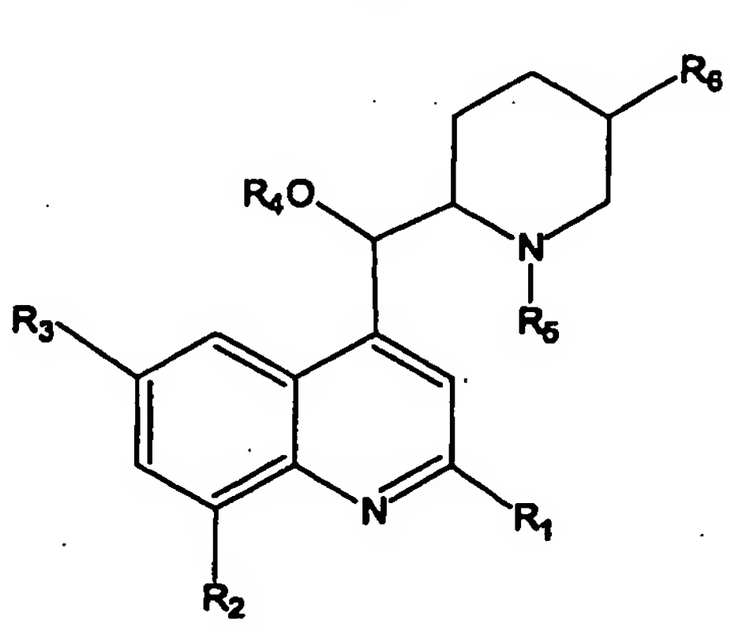
Estrogen and REA also appear to play a role in pathological processes in the eye: Despite the high prevalence of age-related cataracts, there are currently no known therapies to delay or prevent their occurrence. Studies in humans and rodent models suggest that estrogen may provide protection against age-related cataracts. The discovery of ocular estrogen receptors (ERs) indicates that estrogen protection may result from direct interactions with its receptors in the eye, instead an indirect consequence from effects on another tissue. (Davis V. L. et al., Proc. Natl. Acad. Sci. USA 99, 9427-9432, (2002)).

According to the results described in the examples of the present application it was found that mefloquine, nelfinavir and saquinavir and related compounds interact with prohibitin and estrogen receptor repressor (REA) and inhibit VDAC-1. Based on these results it is demonstrated that mefloquine, nelfinavir and saquinavir and related compounds have cytoprotective and particularly neuro-protective efficacy. Since a common feature of the different cellular challenges described in the examples of the present application is an initial cytotoxic calcium overload which subsequently leads to inflammatory and apoptotic events, mefloquine, nelfinavir and saquinavir and related compounds are suitable for the prevention and/or treatment of diseases which are caused by, associated with or accompanied by calcium overload and inflammatory and/or apoptotic events.

Thus, a first aspect of the present invention relates to the use of a compound of formulae (1), (2), (3), (4) or (5)



(1)



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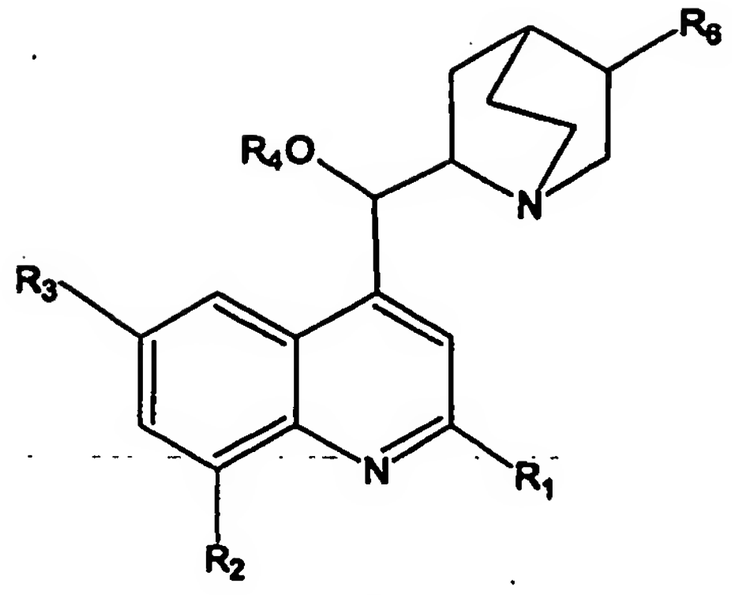

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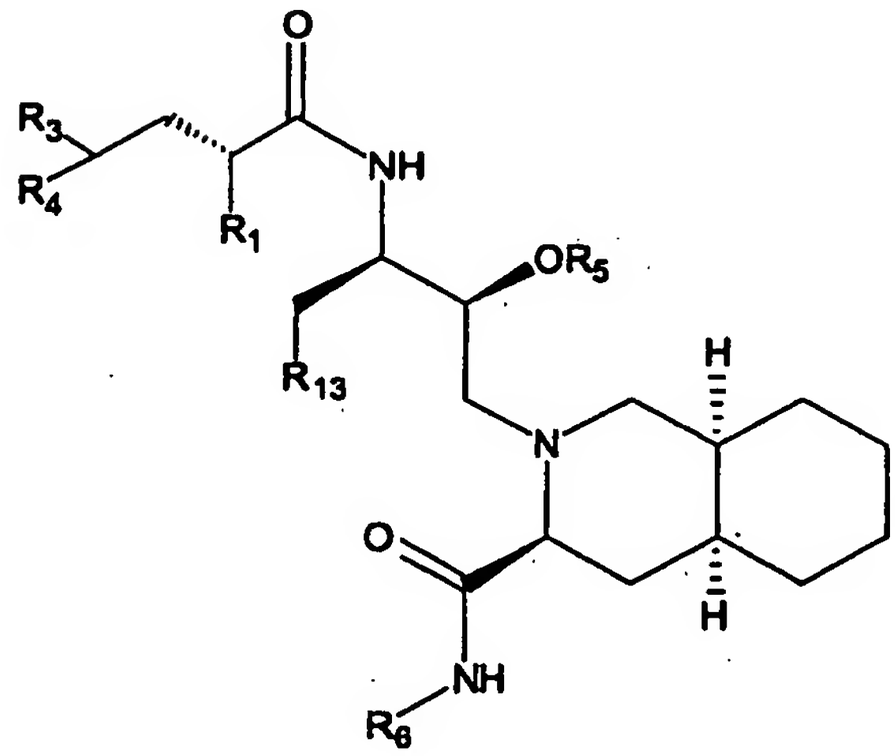

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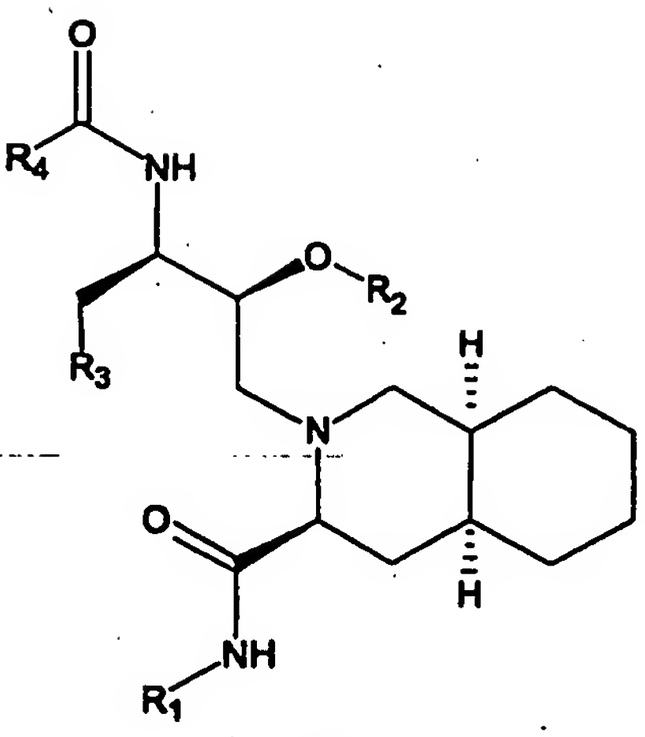


(5)

or an optical isomer, a salt or derivative thereof for the manufacture of a cytoprotective medicament, particularly a medicament for the prevention or

treatment of a disease associated with an inflammatory component, e.g. a neurological or non-neurological inflammatory disease.

In the compounds of the formula (1)

R_1, R_2 and R_4 are independently from each other hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, wherein the alkyl or cycloalkyl group is optionally substituted with a five- or six-membered ring optionally containing at least one heteroatom selected from N, S and O, and wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl, C_1-C_4-(halo)alkoxy, amino, C_1-C_4-alkylamino, di(C_1-C_4-alkyl)amino or Z, wherein Z is a C_1-C_6-(halo)alkyl group ω-substituted with a group $-NR_5R_6$, wherein R_5 and R_6 are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_5 and R_6 together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl and C_1-C_4(halo)alkoxy and

R_3 is hydrogen, C_1-C_6-(halo)alkyl, C_3-C_8-(halo)cycloalkyl, or $-NR_7R_8$ wherein R_7 and R_8 are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_7 and R_8 together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl and C_1-C_4-(halo)alkoxy.

In the compounds of the formulae (2) and (3)

R_1 is hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_6-(halo)cycloalkyl, wherein the alkyl or cycloalkyl group is optionally substituted with a five- or six-membered ring optionally containing at least one heteroatom selected from N, S and O, and wherein the ring is optionally mono- or poly-substituted with halo, C_1-C_4-(halo)alkyl, C_1-C_4-(halo)alkoxy, amino, C_1-C_4-alkylamino, di(C_1-C_4-alkyl)amino or Z, wherein Z is a C_1-C_6-(halo)

alkyl group ω-substituted with a group -NR_7R_8, wherein R_7 and R_8 are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_7 and R_8 together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl and C_1-C_4-(halo)alkoxy,

R_2 is hydrogen, halogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, -NR_9R_{10}, wherein R_9 and R_{10} are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_9 and R_{10} together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo) alkyl and C_1-C_4-(halo)alkoxy,

R_3 is hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, halogen, OR_{11}, wherein R_{11} is C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl,

R_4 is hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, CO-C_1-C_8-alkyl,

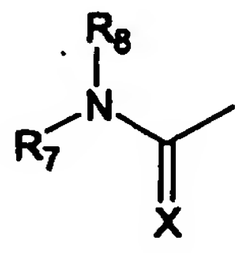
R_5 is hydrogen, C_1-C_6-(halo)alkyl, C_3-C_8-(halo)cycloalkyl or CO-C_1-C_8-alkyl and

R_6 is hydrogen, C_1-C_6-(halo)alkyl, C_3-C_8-(halo)cycloalkyl or C_2-C_6-alkylnyl.

In the compounds of the formula (4)

R_1 is hydrogen, hydroxy or NHR_2,

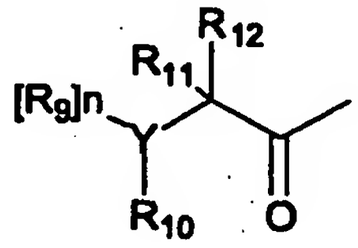
R_2 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heterocyclylalkyl, cycloalkyl, alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, arylalkylcarbonyl, heterocyclylalkylcarbonyl, alkoxycarbonyl, arylalkyl oxycarbonyl, heterocyclylalkoxycarbonyl, aryl, heterocyclyl, sulfonyl, alkylsulfonyl, arylsulfonyl, heterocyclylsulfonyl or a group of the formula



wherein X is O or S and

R_7 and R_8 are independently from each other hydrogen, alkyl, aryl, heterocyclyl, arylalkyl, heterocyclyl alkyl or

R_7 and R_8 together with the nitrogen atom to which they are attached form a saturated ring optionally containing a further heteroatom or a group



wherein R_{10} is hydrogen, alkyl, arylalkyl, heterocyclylalkyl, aryl, heterocyclyl when n=0 and Y is O or S or

R_{10} is hydrogen, alkyl, arylalkyl, heterocyclylalkyl, aryl, heterocyclyl when n=1, Y is N, R_9 is hydrogen or alkyl or

R_9 and R_{10} form together with the heteroatom to which they are attached a heterocyclic ring when n=0 and Y is N, O or S, and

R_{11} and R_{12} independently are hydrogen or alkyl or

R_{11} and R_{12} form together with the carbon atom to which they are attached a ring,

R_3, R_4 are independently from each other hydrogen, alkyl, carbamido, or

R_3, R_4 form together with the carbon atom to which they are attached a carbocyclic ring,

R_5 is hydrogen or the residue of an inorganic or an organic ester and

R_6 is alkyl, arylalkyl, heterocyclylalkyl, alkyloxyalkyl, hydroxyalkyl, amino alkyl, fluoroalkyl and

R_{13} is aryl, ω-alkylaryl, ω-alkylarylether or ω-alkylarylthioether.

In the compounds of the formula (5)

R_1 is alkyl, arylalkyl, heterocyclylalkyl, alkoxyalkyl, hydroxyalkyl, alkylamino, aminoalkyl, fluoroalkyl,

R_2 is hydrogen or the residue of an inorganic or an organic ester,

R_3 is aryl, ω-alkylaryl, ω-alkylaryl ether or ω-alkylaryl thioether and

R_4 is aryl.

The use of mefloquine for the manufacture of a medicament for the prevention or treatment of a disorder which is disclosed in US 6,197,788 is exempted from the use according to the present invention.

The compounds of formulae (1), (2) and (3) and its pharmaceutically acceptable salts may be prepared as described in the citations indicated in the Merck Index, 12th edition, 1996 (cf. citations indicated on page 3 to 4).

The compounds of formulae (4) and (5) and its pharmaceutically acceptable salts may be prepared as described in the state of the art.

The general terms according to the present invention used herein above and below preferably have the following meanings:

"Halo" is, for example, fluorine, chlorine, bromine or iodine.

If not indicated differently, "alkyl" is related to saturated, straight-chain or branched hydrocarbon radicals having 1 to 4, 5, 6, 8, 9 or 10 carbon atoms, e.g. C_1-C_6 alkyl such as methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 1-ethylbutyl, hexyl, 1,1-dimethylpropyl, 1,2- dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl and 1-ethyl-2-methylpropyl.

"C_1-C_8-alkyl" is, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, neopentyl, n-hexyl, n-heptyl or n-octyl, preferably C_1-C_4-alkyl, especially methyl or ethyl, and more especially methyl.

„Alkylcarbonyl" are straight-chain or branched alkyl groups having 1 to 10 carbon atoms (as mentioned above), which are bonded to the structure via a carbonyl group (-CO-).

„Alkylsulfonyl" are straight-chain or branched alkyl groups having 1 to 10 carbon atoms (as mentioned above), which are bonded to the structure via a sulfonyl group ($-SO_2$).

„Alkenyl" are unsaturated, straight-chain or branched hydrocarbon radicals having 2 to 10 carbon atoms and a double bond in any desired position, e.g. C_2-C_6-alkenyl such as ethenyl, 1-propenyl, 2-propenyl.

„Alkylnyl" are straight-chain or branched hydrocarbon groups having 2 to 10 carbon atoms and a triple bond in any desired position, e.g. C_2-C_6-alkynyl.

„(Halo)alkyl" are straight-chain or branched alkyl group having 1 to 4 carbon atoms (as mentioned above) which are optionally substituted by at least one halo, substituent up to perhalogenation.

„Alkoxy" are straight-chain or branched alkyl groups having 1 to 10 or 1 to 10 carbon atoms (as mentioned above), which are bonded to the structure via an oxygen atom (-O-).

„Alkoxycarbonyl" are straight-chain or branched alkoxy groups having 1 to 10 carbon atoms (as mentioned above), which are bonded to the structure via a carbonyl group (-CO-).

„Cycloalkyl" are monocyclic alkyl groups having 3 to 12 carbon ring members, e.g. C_3-C_8-cycloalkyl such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

"Cycloalkylcarbonyl" is a monocyclic alkyl group having 3 to 12, e.g. 3 to 6, 8 to 12 carbon ring members (as mentioned above), which is bonded to the

structure via a carbonyl group (-CO-).

"C_3-C_8-(Halo)cycloalkyl" are monocyclic alkyl groups with 3 to 8 carbon ring members, eg. C_3-C_8-cycloalkyl such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl which are optionally substituted by at least one halo substitutent up to perhalogenation.

"C_1-C_4-(Halo)alkoxy" are straight-chain or linear alkyl groups with 1 to 4, carbon atoms which are bound to the structure by an oxygen atom (-O-) and which are optionally substituted by at least one halo substitutent up to perhalogenation.

"Alkylamino" are straight-chain or branched alkyl groups with 1 to 4 carbon atoms, which are bound to the structure by an amino group (-NH-).

"C_1-C_4-Alkylamino" or "di(C_1-C_4)alkylamino" are straight-chain or branched alkyl groups with 1 to 4 carbon atoms, which are bound to the structure by an amino group (-NH-) or by the nitrogen atom (-N:) respectively.

Aryl or arylalkyl, arylsulfonyl, arylcarbonyl and arylalkoxycarbonyl are aromatic mono- or polycyclic hydrocarbon radicals which are bonded to the structure directly or (arylalkyl) via an alkyl group, (arylsulfonyl) via a sulfonyl group (-SO_2-), (arylcarbonyl) via a carbonyl group (-CO-) and (arylalkoxycarbonyl) via an alkoxycarbonyl group, e.g. phenyl, naphthyl and phenanthryl and the corresponding radicals.

Heterocyclyl is a five- or six-membered ring optionally containing at least one heteroatom selected from N, S and O.

A five- or six-membered ring optionally containing at least one heteroatom selected from N, S and O according to the present invention may be for example:

a 5-membered heteroaryl, containing one to three nitrogen atoms: 5-membered ring heteroaryl groups, which in addition to carbon atoms can contain one to three nitrogen atoms as ring members, eg. 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 4-pyrazolyl, 5-pyrazolyl, 2-imidazolyl, 4-imidazolyl, 1,2,4-triazol-3-yl and 1,3,4-triazol-2-yl;

a 5-membered heteroaryl, containing one to four nitrogen atoms or one to three nitrogen atoms and a sulfur or oxygen atom or an oxygen or a sulfur atom: 5-membered ring heteroaryl groups, which in addition to carbon atoms can contain one to four nitrogen atoms or one to three nitrogen atoms and a sulfur or oxygen atom or an oxygen or sulfur atom as ring members, eg. 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyrrolyl, 3-pyrrolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 3-isothiazolyl, 4-isothiazolyl, 5-isothiazolyl, 3-pyrazolyl, 4-pyrazolyl, 5-pyrazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-imidazolyl, 4-imidazolyl, 1,2,4-oxadiazol-3-yl, 1,2,4-oxadiazol-5-yl, 1,2,4-thiadiazol-3-yl, 1,2,4-thiadiazol-5-yl, 1,2,4-triazol-3-yl, 1,3,4-oxadiazol-2-yl, 1,3,4-thiadiazol-2-yl, 1,3,4-triazol-2-yl;

a fused 5-membered heteroaryl, containing one to four nitrogen atoms or one to three nitrogen atoms and/or an oxygen or sulfur atom: 5-membered ring heteroaryl groups, which in addition to carbon atoms can contain one to four nitrogen atoms or one to three nitrogen atoms and a sulfur or oxygen atom or an oxygen or a sulfur atom as ring members, and in which two adjacent carbon ring members or a nitrogen and an adjacent carbon ring member can be bridged to form an aromatic or heteroaromatic bicycle or polycycle, eg. benzofuranyl, isobenzofuranyl, benzothienyl, isobenzothienyl, indolyl, isoindolyl, benzoisoxazolyl, benzoxazolyl, benzoisothiazolyl, benzothiazolyl, indazolyl, benzimidazolyl, pyrrolopyridinyl, pyrrolopyridazinyl, pyrrolopyrimidinyl, pyrrolopyrazinyl, pyrrolotriazinyl, furopyridinyl, furopyridazinyl, furopyrimidyl, furopyrazinyl, furotriazinyl, thienopyridinyl, thienopyridazinyl, thienopyrimidyl, thienopyrazinyl, thienotriazinyl, imidazopyridinyl, imidazopyridazinyl, imidazopyrimidyl, imidazopyrazinyl, imidazotriazinyl, pyrazolopyridinyl, pyrazolopyridazinyl, pyrazolopyrimidyl,

pyrazolopyrazinyl, pyrazolotriazinyl, isoxazolopyridinyl, isoxazolopyridazinyl, isoxazolopyrimidyl, isoxazolopyrazinyl, isoxazolotriazinyl, oxazolopyridinyl, oxazolopyridazinyl, oxazolopyrimidyl, oxazolopyrazinyl, oxazolotriazinyl, isothiazolopyridinyl, isothiazolopyridazinyl, isothiazolopyrimidyl, isothiazolopyrazinyl, isothiazolotriazinyl, thiazolopyridinyl, thiazolopyridazinyl, thiazolopyrimidyl, thiazolopyrazinyl, thiazolotriazinyl, triazolopyridinyl, triazolopyridazinyl, triazolopyrimidyl, triazolopyrazinyl and triazolotriazinyl;

a 6-membered heteroaryl, containing one to three or one to four nitrogen atoms: 6-membered ring heteroaryl groups, which in addition to carbon atoms can contain one to three or one to four nitrogen atoms as ring members, eg. 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, 3-pyridazinyl, 4-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 2-pyrazinyl, 1,3,5-triazin-2-yl, 1,2,4-triazin-3-yl and 1,2,4,5-tetrazin-3-yl; or

an aliphatic heterocyclyl such as piperidinyl and morpholinyl.

Heterocyclylalkyl, Heterocyclylsulfonyl, Heterocyclylcarbonyl and Heterocyclylalkoxycarbonyl are aromatic mono- or polycyclic hydrocarbon radicals which in addition to carbon ring members additionally can contain one to four nitrogen atoms or one to three nitrogen atoms and an oxygen or a sulfur atom or an oxygen or a sulfur atom and which are bonded to the structure (heterocyclylalkyl) via an alkyl group, (heterocyclylsulfonyl) via a sulfonyl group (-SO_2-), (heterocyclylcarbonyl) via a carbonyl group (-CO-), and (heterocyclylalkoxycarbonyl) via an alkoxycarbonyl group.

The compounds of formulae (1), (2), (3), (4) and (5) may be present as an optical enantiomer or diastereomer or any mixture of enantiomers, such as racemates, or diastereomers.

The term „salt" preferably refers to pharmaceutically acceptable salts of compounds of formulae (1), (2), (3), (4) and (5) with suitable cations and/or

anions. Examples of suitable cations are alkaline metal cations such as Li^+; Na^+ and K^+, alkaline earth metal cations such as Mg^{2+} and Ca^{2+} as well as suitable organic cations, e.g. ammoniums or substituted ammonium cations. Examples of pharmaceutically acceptable anions are inorganic anions such as chloride, sulfate, hydrogensulfate, phosphate or organic anions such as acetate, citrate, tartrate, etc.

Derivatives of the compounds of formulae (1), (2), (3), (4) and (5) are any molecules which are converted under physiological conditions to a compound of formulae (1), (2), (3), (4) and (5) respectively, e.g. esters, amides etc. of compounds of formulae (1), (2), (3), (4) and (5) molecules which are products of metabolization reactions of a any compound of formulae (1), (2), (3), (4) and (5).

Preferred examples of the compounds of formulae (1), (2) and (3) include mefloquine and derivatives thereof, e.g. acetyl mefloquine, chloroquine, quenine, primquine, ablaquine and amodiaquine as well as derivatives thereof.

Preferred examples of the compounds of formulae (4) and (5) include nelfinavir/saquinavir and derivatives thereof, e.g. amprenavir (Agenerase®), indinavir (Crixivan®), lopinavir (Kaletra®), ritonavir (Norvir®) and atazanavir (Reyataz®).

Preferably, the compounds of formulae (1) to (5) are used for the prevention or treatment of disorders associated with apoptotic or neuroinflammatory, or generally inflammatory disorders which are caused by and/or accompanied by mitochondrial dysfunction, particularly a dysfunctional mitochondrial transition pore, and/or disorders which are caused by and/or accompanied by HDAC-dysfunction, particularly a dysfunctional decrease in HDAC activity.

For example these disorders include neurodegenerative or

neuroinflammatory diseases or pathological conditions, like M. Alzheimer, traumatic brain injury, M. Parkinson, amytrophic lateral sclerosis (ALS), stroke, migraine and multiple sclerosis.

Also, at least one of the common lung diseases associated with a significant inflammatory component such as severe sepsis, acute lung injury, acute respiratory distress syndrome, cystic fibrosis, asthma, allergic rhinitis, or COPD or lung cancer, as well as any other cancer type.

A further preferred indication is the prevention or treatment of cystic fibrosis, particularly in persons with impaired function of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel or the prevention or treatment of ulcerative or other inflammatory conditions of the gastrointestinal system, particularly of persons with impaired function of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel.

Still a further preferred indication is for the prevention or treatment of inflammatory processes involved in cancer prevention or progression.

Still a further preferred indication is for the prevention or treatment of inflammatory processes involved in autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus, especially for the treatment of patients identified by genetic or other markers to be more likely to be especially amenable to such treatment.

Still a further indication is for the prevention and treatment of pain, particularly inflammatory or neurological pain, because it has been shown that proapoptotic mechanisms play a role in initial phases of various forms of chronic pain (Maione S et al., Apoptotic genes expression in the lumbar dorsal horn in a model neuropathic pain in rat. Neuroreport 2002 Jan 21;13 (1):101-6).

Still, a further preferred indication is for the prevention or treatment of

inflammatory or inflammation-associated ocular disorders, particularly macula degeneration, e.g. moist or dry macula degeneration, glaucoma, e.g. acute, primary, secondary or low angle glaucoma, diabetic retinopathy, anterior or posterior optical neuropathy, retinitis pigmentosa, neuritis nervi optici, or central artery obstruction.

A further aspect of the present invention relates to the use of a compound which is a MTP or REA inhibitor/modulator for the manufacture of a medicament for the prevention or treatment of airway diseases, preferably for the prevention or treatment of disorders as indicated above.

For therapeutic applications, the compounds of formulae (1) to (5) may be used alone or together with other medicaments, e.g. oral asthma medications, antidiabetic or anticancer treatments.

The dual inhibitor compound is preferably a moderately strong VDAC-1 inhibitor, which has an IC_{50} value for VDAC from 100 to 10000 µM, more preferably from 250 to 1000 µM. The determination of the IC_{50} value is carried out as indicated as in the Examples.

Still, a further aspect of the present invention relates to the use of a compound which is a dual VDAC-1/REA inhibitor or binding protein for the manufacture of a neuro- or cytoprotective medicament, preferably for the prevention or treatment of disorders as indicated above.

The compound is preferably a moderately strong VDAC-1 inhibitor as indicated above. Further, the IC_{50} value is from 1 to 10,000 µM, more preferably from 5 to 1,000 µM. The determination of the IC_{50} value is carried out as indicated in the Examples.

The compounds as indicated above are preferably administered to a subject in need thereof as a pharmaceutical composition, which may contain

pharmaceutically acceptable carriers, diluents and/or adjuvants. The pharmaceutical composition may be administered in the form of a tablet, capsule, solution, suspension, aerosol, spray etc (e.g. nasal or throat spray), gel, plaster etc. The medicament may be administered according to any known means, wherein oral, pulmonal and intravenous administration is particularly preferred. The dose of the active ingredient depends on the type and the variety of disease and usually is in the range from 1 to 2000 mg/day, preferably in the range from 10 to 200 mg/day.

The present application has applications in human and veterinary medicine, particularly in human medicine.

Furthermore, the present invention shall be explained by the following Figures and examples.

Figure legends

Fig. 1: Neuroprotective effect of mefloquine in the functional model outlined in the methods section.

Fig. 2: Neuroprotective effect of acetylmefloquine in the functional model outlined in the methods section.

Fig. 3: Silver stained gels of fractions enriched with Mefloquine-based affinity reagent (M: markers; RE: raw extract; F1-2, flow throughs, proteins not binding to affinity beads; E1-3: proteins binding to affinity beads and recovered by elutions of varying stringency): the numbered bands were identified by mass spectrometry. The proteins in the specific mefloquine-associated fractions E1-3, are those with numbers 41-54: Beyond some structural and housekeeping proteins (e.g. tubulin isoforms), considered to be associated , but rather for anchoring than drug binding the following bands appeared to be associated with mefloquine binding: 45, 46 and 48; names and gene bank accession numbers are given in the following table:

MW_{app} is the apparent molecular weight, pI is measured isoelectric point on 2D gels; MW_{cal} is the calculated molecular weight.

Fig. 4: Structure of Nelfinavir-Sepharose 4B.

Fig. 5: Neuroprotective effect of nelfinavir and saquinavir in the functional model outlined in the methods section at concentrations of each 1 µM.

Fig. 6: Silver stained gels of fractions enriched with Nelfinavir/Saquinavir-based affinity reagent (1,2: raw extracts; 3-9, flow throughs, unspecific elutions, proteins not related to nelfinavir-part of affinity material beads; 10,11: proteins binding to affinity beads and containing specific nelfinavir-related bands): the numbered bands were identified by mass spectrometry. The proteins in the specific Nelfinavir/Saquinavir-associated fractions E1-3, are those with numbers 14-19: Beyond some structural and housekeeping proteins (e.g. tubulin isoforms), considered to be associated , but rather for anchoring than drug binding, the following bands appeared to be associated with Nelfinavir/Saquinavir binding: 14-10; names and gene bank accession numbers are given in the following table:
MW_{exp} is the apparent molecular weight, pI is measured isoelectric point on 2D gels; MW_{theor} is the calculated molecular weight.

Examples

Example 1

Using a functional cellular model of neuroprotection and a set of neuronal biomarkers a screening of test compounds for novel neuroprotective modes of action was carried out (Sommer S. et al., J. Proteome Res. 3, 572-581, (2004); Schrattenholz A. et al., J. Neurol. Sci., in press (2005)). Surprisingly, it was found that mefloquine and related compounds have a previously unknown and neuroprotective mode of action via binding to VDAC-1, REA and prohibitin. Due to these previously unknown neuroprotective effects, the

compounds are suitable as cytoprotective drugs and new lead structures for the development and optimization of related compounds with this novel mode of action, generally for cytoprotection and, particularly for the treatment of disorders of the nervous system related to underlying apoptotic and/or inflammatory mechanisms.

1. Materials and Methods

1.1 Biological test system; Cell culture model for chemical ischemia and neuroprotection

For all experiments, D3 embryonic stem (ES) cells derived from 129/sv mice [Okabe et al., Mech. Dev. 59, 89-102 (1996)] were cultivated for 12 days, with passages on days 2, 4, 7 and 9 as described previously (Sommer S. et al., supra 2004). Insult conditions: Cells (24-well plates) were pre-incubated with or without 20nM EPO in fresh medium for 24 hours at 37°C. Cells were rinsed once with low K^+ solution (140 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 11 mM glucose, 15 mM Hepes-NaOH, pH 7.35). Cells (either with or without EPO pre-incubation) were incubated for up to 45 min (37°C) with either low K^+ solution or with glucose-free low K^+ solution supplemented with 1mM KCN (chemical ischemia solution, Kume et al., Eur J Pharmacol. 455, 91-100 (2002)). Vitality control to assess numbers of surviving neurons was performed by a brief stimulation with a low dose of glutamate (10 µM). Afterwards, cells were washed three times with ice cold phosphate buffered saline (PBS), and then proteins were harvested. Suspended cells were pelleted at 500 x G, and lysed into 9M urea 4% CHAPS. The cell lysate was desalted with a NAP-10 column (Amersham Biosciences), preequilibrated with the same buffer, and protein content was determined.

1.2 Calcium-Imaging

Functional tests by calcium imaging were performed essentially as described

(Sommer S. et al., supra (2004)). Briefly, cells were loaded with 2 µM of fura-2 AM in DMEM for 45 min at 37°C in the dark. Measurements of relative changes in [Ca^{2+}] were made on an inverted epifluorescence microscope (Olympus IX70 S1F2) with a Polychrom IV Monochromator (Xe-lamp, USHIO). Excitation wavelengths (λ_1, λ_2) and the emission wavelength were 340, 380 and 510 nm, respectively. Acquisition and analysis of data after appropriate stimulation were performed by using MetaFluor software (Universal Imaging Corporation). Image resolution was 168 x 129 pixels (binning 8 x 8, pixel size 6.8 x 6.8 µm). Only cells identified as neurons by morphological criteria and occasional immunostaining (not shown) and those whose calcium levels returned to the resting state after the first stimulation were taken into account. Controls included nominal zero calcium (negative) and 5 µM ionomycin (positive), 10 µM glutamate (positive) and depolarisation (55 mM K^+) (positive). Pharmacological agents were applied by a multi-valve, single-output focal drug application device (ALA Scientific) with the perfusion system DAD-12. Ratio images were displayed as a percentage of relative change in fluorescence over background fluorescence scale for comparison across experiments (as described in Sommer S. et al., supra (2004)). During each stimulation event, 20 image pairs were acquired.

1.3 Chemical Proteomic - synthesis of mefloquine-AffiGel 10

To a solution of mefloquine HCl (200 mg) prepared in 10 ml DMF and 0.3 ml N-methylmorpholine, 10 ml of a 50% suspension of AffiGel 10 (BioRad) in isopropanol was added. The suspension was shaken at room temperature for 16 hours whereupon 0.3 ml of ethanolamine was added. After another 2 hours of shaking at room temperature beads were separated by filtration through glass sinter funnel and washed sequentially with 200 ml of DMF/isopropanol (1/1), 200 ml isopropanol, 200 ml 60% isopropanol, 500 ml

30% isopropanol and 1000 ml water. The obtained mefloquine-AffiGel 10 beads were kept as 50% slurry in 30% isopropanol at 4 °C until used.

1.4 Identification and characterization of second binding sites of mefloquine and derivatives

The affinity beads (Meloquine-Affi-Gel 10) were used to bind the target(s) from fractionated crude cell extracts of D3 ES cells and other cell lines. Subsequently the affinity purified material was analysed by 1D PAGE, immunostaining, and mass spectrometry.

1.4.1 Fractionation, isolation, Western blots, mass spectrometry

The subsequent fractionation, isolation and further analysis was performed according to published procedures (Sommer S. et al., supra, (2004)). Mass spectrometry for independent identification of mefloquine-tagged proteins was performed as described elsewhere recently (Vogt J. A. et al., Rapid Commun. Mass Spectrom. 17, 1273-1282, (2003) and Anal. Chem. 77, in press, (2005), Cahill M. A. et al., Rapid Commun. Mass Spectrom. 17, 1283-1290, (2003)).

Monoclonal anti-PARP antibody was purchased from BD BioScience (Cat# 556 362; clone C2-10). Secondary anti-mouse alkaline phosphatase conjugate was purchased from Sigma (Cat# A9316). NBT/BCIP-westernblot detection reagents came from Roche Diagnostics (Cat.# 1681451), Western Lightening CDP-Star chemiluminescence detection kit was supplied by Perkinelmer (Cat.# NEL616001KT). For anti-PARP western blotting experiments proteins were separated on 10% polyacryl amide gels and blotted onto nitrocellulose. Blots were blocked with 5% skimmed milk powder in Tris buffered saline containing 0,1% Tween-20 (TBS-T). anti-PARP antibody was incubated over night at 4°C using a 1:1000 dilution in milk powder TBS-T. Blots were subsequently washed 3 times using TBS-T. A second antibody was used at a dilution of 1:1000 for NBT/BCIP detection

and 1:5000 for CDP-Star detection.

Cox-2 staining was obtained accordingly by using an antibody from Alexis, (ALX-210-711-1) anti-COX-2 (Cyclooxygenase-2); Rabbit, polyclonal; 1:1000 dilution; secondary antibody was anti-rabbit-AP (Sigma, A3937, 1:1000)

iNOS staining was performed using a polyclonal anti-iNOS, Alexis, 1:1000). Blots were washed in TBS/1.0 % Tween and incubated with the appropriate secondary antibody-horseradish peroxidase conjugate (anti-rabbit IgG, Sigma, 1:2000).

1.4.2 HDAC-1 activity assay

For measurements of HDAC activities, the quantitative test kit for NAD-dependent histone deacetylase activity CycLex® Sir2 Assay kit (Cat# CY-1151) was used according to instructions of the manufacturer (CycLex Co., Ltd. 1063-103 Ohara, Tera-Sawaoka Ina, Nagano 396-0002 Japan). All substances tested in the SIR assay have to be cross-checked for their influence on the lysyl-endopeptidase. For this control an already deacetylated substrate peptide is used in order to measure directly lysyl-endopeptidase activity.

1.4.3 LPS experiments

LPS challenge of 3T3 fibroblasts, A 549 cells, V56 embryonic stem cells and neurally differentiated V56 embryonic stem cells was equally performed by exposing cells to 100 ng/ml lipopolysaccharide (LPS, E.coli 0111:B4 LPS from Sigma) for 60 min in the presence or absence of mefloquine and related compounds. Cell pellets were further investigated by Western blot staining with anti cox-2 and anti iNOS antibodies of 1D polyacrylamide gels.

1.4.4 ADP-ribosylated proteins

Poly-ADP ribosylated proteins were quantified from appropriate cellular

protein extracts by Western blots using as primary antibody: Anti-poly(ADP-ribose); Antigen: mouse; Biomol, Cat Nr SA-216, Lot Nr. : P7482; and as secondary antibody: Anti mouse, AP; Sigma A 9316, Lot: 31K 9205; both antibodies were used at 1:2000 dilution.

1.4.5 AIF kinetics, Western blots

For quantification of release of apoptosis inducing factor (AIF) from mitochondria after ischemic/excitotoxic or β-amyloid related insults the cellular material was fractionated into cytosolic, nuclear and mitochondrial fractions according to Arnoult et al. (Damien Arnoult, Philippe Parone, Jean-Claude Martinou, Bruno Antonsson, Jérôme Estaquier, and Jean Claude Ameisen Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome *c* release in response to several proapoptotic stimuli Journal of Cell Biology, Volume 159, Number 6, December 23, 2002 923–929).

Staining of related Western blots was performed using as primary antibody: anti AIF (apoptosis inducing factor); Antigen: goat polyclonal; Sc-9416 from Santa Cruz; at a dilution of 1:2000; and as secondary antibody anti-goat IgG, whole molecule; developed in rabbit, A 4174 Sigma , peroxidase-coupled at a dilution of 1:1000.

2. Results

2.1. Neuroprotection in vitro

Neuroprotective effect of mefloquine in chemical ischemia, excitotoxic and β-amyloid-induced neuronal insult

Figure 1 shows the neuroprotective effect of mefloquine in the functional model outlined in the methods section and Figure 2 those of its derivative acetylmefloquine.

Whereas control cells had a survival rate of 28.3 ± 1.8%, mefloquine-treated cells had survival rates ranging from of 47.1 to 61.3 % with an EC50 of approx. 90 nM. As a summary of neuroprotective effects of mefloquine and and acetyl-mefloquine in three different insults the following was observed: After induction of chemical ischemia as described we found an EC 50 value of about 90 nM, after induction of excitotoxic cell death by 100 µM NMDA (or 100 µM HCA as in Sommer S. et al., supra (2004)), we found EC 50 value of 200 nM and after induction of neuronal death by 10 µM β-amyloid1-40 (Bachem, Germany), we found an EC 50 value of 100 nM; All three insults induce an initial calcium overload, which obviously initiates proapoptotic and proinflammatory events, leading eventually to neuronal dysfunction and cell death. A decrease of apoptotic and inflammatory markers PARP-1, AIF, cox-2 and iNOS during neuroprotective treatment with mefloquine and acetylmefloquine in the in vitro model was found.

2.2 Chemical Proteomics

2.2.1 Identification of VDAC-1, REA and prohibitin as targets of mefloquine

We then proceeded to synthesize reactive mefloquine derivatives. Mefloquine was used as a starting structure for the synthesis of the affinity reagent, which was used to bind the targets from fractionated crude cell extracts of D3 embryonic stem cells. Subsequently the affinity purified material was analysed by 1D PAGE (Figure 3) and mass spectrometry. MALDI-TOF analysis of the silver stained gels indicated the presence of VDAC-1, REA and prohibitin in enriched fractions.

2.2.2 Electrophysiological VDAC-1 Assay

cDNAs for Homo sapiens prohibitin (PHB) and Homo sapiens voltage-dependent anion channel 1 (VDAC-1) were purchased from Origene

Technologies Inc. USA. VDAC-1 was expressed in Xenopus laevis oocytes, alone and together with prohibitin. Electrophysiological measurements show that mefloquine and acetylmefloquine are inhibitors of VDAC-1 and that prohibitin is modulating this inhibitory effect.

2.2.3 REA assay

The possibility that REA interacts with histone deactylases (HDAC) prompted us to test mefloquine and derivatives in a quantitative test kit for NAD-dependent histone deacetylase activity (CycLex Assay kit): The substances appear to be inhibitors of HDAC activity.

2.3 General cytoprotective effects towards an inflammatory insult (LPS exposure)

We stimulated 3T3 fibroblasts, A549 cells, undifferentiated V56 embryonic stem cells and neurally differentiated V56 embryonic stem cells with 100 ng/ml lipopolysaccharide (E.coli 0111:B4 LPS from Sigma) for 60 min. As an inflammatory marker we again quantified expression of cox-2 and iNOS by appropriate antibody staining of Western blots of 1D PA gels. The results show that mefloquine and related substances like LS-75 i) protect cells from LPS-induced death, and ii) that this protective effect is accompanied by a decreased expression of inducible inflammatory markers iNOS and cox-2. Cell survival was assessed by Trypan Blue staining.

2.4 Influence/dependence of effects of mefloquine and related substances upon assembly of cholesterol-rich membrane domains

Next to the direct effect on VDAC-1, REA and prohibitin, the substances appear to bring about their effects via transient membrane domains, cholesterol-rich lipid rafts, which are thought to be an important in a variety of related signalling pathways (Cuschieri J. Implications of lipid raft disintegration: enhanced anti-inflammatory macrophage phenotype.

Surgery. 2004 Aug;136(2):169-75.; Chu CL, Buczek-Thomas JA, Nugent MA. Heparan sulphate proteoglycans modulate fibroblast growth factor-2 binding through a lipid raft-mediated mechanism. Biochem J. 2004 Apr 15;379(Pt 2):331-41; Argyris EG, Acheampong E, Nunnari G, Mukhtar M, Williams KJ, Pomerantz RJ.Human immunodeficiency virus type 1 enters primary human brain microvascular endothelial cells by a mechanism involving cell surface proteoglycans independent of lipid rafts. J Virol. 2003 Nov;77(22):12140-51; Nagy P, Vereb G, Sebestyen Z, Horvath G, Lockett SJ, Damjanovich S, Park JW, Jovin TM, Szollosi J. Lipid rafts and the local density of ErbB proteins influence the biological role of homo- and heteroassociations of ErbB2. J Cell Sci. 2002 Nov 15;115(Pt 22):4251-62).

2.5 Conclusions

Our results clearly show, that mefloquine and related compounds act on mitochondrial targets mentioned above.

This property of mefloquine and related compounds was previously unknown and allows the conclusion that mefloquine and related compounds may be used as cytoprotective agents for medical applications. Due to this novel mode of action which is different from the previously described (Foley M. et al., Int. J. Parasitol. 12, 1211-23 (1997)) we see an opportunity for new development strategies.

The cytoprotective properties of these and other related compounds are rather due to a hitherto unknown dual mode of action namely VDAC-1 and REA. This novel mixed type of activity can be used for new high throughput screening of existing chemical libraries for identification of novel cytoprotective agents for the treatment of various indications as outlined above.

Generally the invention relates to cytoprotective properties of compounds with a VDAC-1 modulating activity.

3. Cox-2 and iNOS expression in LPS challenge and chemical ischemia of neuronal and non-neuronal cells

In the various cellular insults described here, we always observe an initial calcium overload of cells, which subsequently leads to apoptotic cell death, concomitant with increase of apoptotic and proinflammatory markers.

4. General conclusions

We see here neuro- and more generally cytoprotective effects of mefloquine and related compounds which on the one hand appear to be mediated via mitochondrial mechanisms involving prohibitin and REA, but in particular by VDAC-1 binding and inhibition, and on the other hand appear to require a special assembly of membrane associated protein complexes in so-called lipid rafts.

Nevertheless, one common feature of all the different cellular challenges applied here in the context of said substances is an initial cytotoxic calcium overload, which subsequently proceeds to inflammatory and apoptotic events as demonstrated by PARP-1/iNOS/cox-2 staining. Thus we claim the use of said substances as treatment in all disease indications where calcium overload and inflammatory/apoptotic events are thought to play a major role or potentially are crucial. This includes Alzheimer's and Parkinson's disease, traumatic brain injury, ALS, multiple sclerosis, migraine and chronic pain syndromes. (Other non-neuronal diseases mentioned above).

Still, a further preferred indication is for the prevention or treatment of inflammatory or inflammation-associated ocular disorders, particularly macula degeneration, e.g. moist or dry macula degeneration, glaucoma, e.g. acute, primary, secondary or low angle glaucoma, diabetic retinopathy, anterior or posterior optical neuropathy, retinitis pigmentosa, neuritis nervi optici, or central artery obstruction.

Example 2

Using a functional cellular model of neuroprotection and a set of neuronal biomarkers a screening of test compounds for novel neuroprotective modes of action was carried out (Sommer S. et al., J. Proteome Res. 3, 572-581, (2004); Schrattenholz A. et al., J. Neurol. Sci., in press (2005)). Surprisingly, it was found that nelfinavir, saquinavir and related compounds have a previously unknown and neuroprotective mode of action via binding to VDAC-1, REA and prohibitin. Due to these previously unknown neuroprotective effects, the compounds are suitable as cytoprotective drugs and new lead structures for the development and optimization of related compounds with this novel mode of action, generally for cytoprotection and, particularly for the treatment of disorders of the nervous system related to underlying apoptotic and/or inflammatory mechanisms.

1. Materials and Methods

1.1 Biological test system; Cell culture model for chemical ischemia and neuroprotection

For all experiments, D3 embryonic stem (ES) cells derived from 129/sv mice [Okabe et al., 1996, supra] were cultivated for 12 days, with passages on days 2, 4, 7 and 9 as described previously (Sommer S. et al., supra 2004). Insult conditions: Cells (24-well plates) were pre-incubated with or without 20nM EPO in fresh medium for 24 hours at 37°C. Cells were rinsed once with low K^+ solution (140 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 11 mM glucose, 15 mM Hepes-NaOH, pH 7.35). Cells (either with or without EPO pre-incubation) were incubated for up to 45 min (37°C) with either low K^+ solution or with glucose-free low K^+ solution supplemented with 1mM KCN (chemical ischemia solution, Kume et al., 2002, supra). Vitality control to assess numbers of surviving neurons was performed by a brief stimulation with a low dose of glutamate (10 µM).

Afterwards, cells were washed three times with ice cold phosphate buffered saline (PBS), and then proteins were harvested. Suspended cells were pelleted at 500 x G, and lysed into 9M urea 4% CHAPS. The cell lysate was desalted with a NAP-10 column (Amersham Biosciences), preequilibrated with the same buffer, and protein content was determined.

1.2 Calcium-Imaging

Functional tests by calcium imaging were performed essentially as described (Sommer S. et al., supra (2004)). Briefly, cells were loaded with 2 µM of fura-2 AM in DMEM for 45 min at 37°C in the dark. Measurements of relative changes in $[Ca^{2+}]$ were made on an inverted epifluorescence microscope (Olympus IX70 S1F2) with a Polychrom IV Monochromator (Xe-lamp, USHIO). Excitation wavelengths (λ_1, λ_2) and the emission wavelength were 340, 380 and 510 nm, respectively. Acquisition and analysis of data after appropriate stimulation were performed by using MetaFluor software (Universal Imaging Corporation). Image resolution was 168 x 129 pixels (binning 8 x 8, pixel size 6.8 x 6.8 µm). Only cells identified as neurons by morphological criteria and occasional immunostaining (not shown) and those whose calcium levels returned to the resting state after the first stimulation were taken into account. Controls included nominal zero calcium (negative) and 5 µM ionomycin (positive), 10 µM glutamate (positive) and depolarisation (55 mM K^+) (positive). Pharmacological agents were applied by a multi-valve, single-output focal drug application device (ALA Scientific) with the perfusion system DAD-12. Ratio images were displayed as a percentage of relative change in fluorescence over background fluorescence scale for comparison across experiments (as described in Sommer S. et al., supra (2004)). During each stimulation event, 20 image pairs were acquired.

1.3 Chemical Proteomics - Synthesis of Nelfinavir/Saquinavir-Sepharose 4B

1.3.1 Synthesis of p-Nitrobenzoylamido Sepharose 4B

250 mg of p-nitrobenzoyl chloride was dissolved in 1 ml DMF. This solution was mixed with 25% suspension of amino-Sepharose 4B (20 ml) in 60% iPrOH/ 1.0 M $KHCO_3$ (2/1). The reaction was performed at room temperature for 1 hour. Sepharose was separated by filtration and resuspended again in the same medium and treatment with p-nitrobenzoyl chloride was repeated. The reaction was performed at room temperature for another 2 hours. where upon obtained beads were washed with 60% 2-propanol (200 ml), 30 % iPrOH (200 ml) and water (500 ml).

1.3.2 Synthesis of p-Nitrobenzoylamido Sepharose 4B

1000 mg of sodium dithionite was dissolved in 20 ml 1M $NaHCO_3$. This solution was mixed with 5 g sucking dried p-nitrobenzoylamido Sepharose 4B. The reaction was performed at room temperature for 3 hours. Sepharose was separated by filtration and resuspended again in 0.2 M HCl at 4 °C.

1.3.3 Synthesis of p-Diazoniumbenzoylamido Sepharose 4B:

120 mg of sodium nitrite was dissolved in 1 ml of water. This solution was mixed with suspension 5 g sucking dried p-aminobenzoylamido Sepharose 4B in 30 ml 0.2 M HCl. The reaction was performed on ice bath temperature for 15 min. p-Diazobenzoylamido Sepharose 4B was separated by filtration and washed with ice in 0.1 M HCl (200 ml), 0.01 M HCl (200 ml) and 50% DMF. It was used immediately for preparation of of p-diazoniumbenzoylamido Nelfinavir-Sepharose 4B.

1.3.4 Synthesis of Nelfinavir Sepharose 4B:

320 mg of nelfinavir (free base or saquinavir) was dissolved in 20 ml of 90% DMF and 200 µl of ethyldiisopropylamine was added. The solution was

cooled on ice. This solution was mixed with a suspension of 5 g sucked dried p-diazoniumbenzoylamido Sepharose 4B and shaked for 2 hours at room temperature. Then 0.2 ml of aniline was added and shaking was continued for another 60 min. Nelfinavir Sepharose 4B was separated by filtration and washed with 80% DMF (200 ml), 40% DMF (200 ml), water (200 ml), 0.1 M HCl (200 ml) water 500 ml. Finally it was resuspended in 30% 2-propanol and kept in fridge until used.

The structure of Nelfinavir-Sepharose 4B is depicted in Figure 4.

The following examples relate to nelfinavir. The approach was adapted accordingly for saquinavir, yielding absolutely similar results.

1.4 Identification and characterization of second binding sites of nelfinavir/saquinavir and derivatives

The affinity beads (nelfinavir/saquinavir-Affi-Gel 10) were used to bind the target(s) from fractionated crude cell extracts of D3 ES cells and other cell lines. Subsequently the affinity purified material was analysed by 1D PAGE, immunostaining, and mass spectrometry.

1.4.1 Fractionation, isolation, Western blots, mass spectrometry

The subsequent fractionation, isolation and further analysis were performed according to published procedures (Sommer S. et al., supra, (2004)). Mass spectrometry for independent identification of nelfinavir-tagged proteins was performed as described elsewhere recently (Vogt J. A. et al., Rapid Commun. Mass Spectrom. 17, 1273-1282, (2003) and Anal. Chem. 77, in press, (2005), Cahill M. A. et al., Rapid Commun. Mass Spectrom. 17, 1283-1290, (2003)).

Monoclonal anti-PARP antibody was purchased from BD BioScience (Cat# 556 362; clone C2-10). Secondary anti-mouse alkaline phosphatase conjugate was purchased from Sigma (Cat# A9316). NBT/BCIP-westernblot detection reagents came from Roche Diagnostics (Cat.# 1681451), Western Lightening CDP-Star chemiluminescence detection kit was supplied by Perkinelmer (Cat.# NEL616001KT). For anti-PARP western blotting experiments proteins were separated on 10% polyacryl amide gels and blotted onto nitrocellulose. Blots were blocked with 5% skimmed milk powder in Tris buffered saline containing 0,1% Tween-20 (TBS-T). anti-PARP antibody was incubated over night at 4°C using a 1:1000 dilution in milk powder TBS-T. Blots were subsequently washed 3 times using TBS-T. A second antibody was used at a dilution of 1:1000 for NBT/BCIP detection and 1:5000 for CDP-Star detection.

Cox-2 staining was obtained accordingly by using an antibody from Alexis, (ALX-210-711-1) anti-COX-2 (Cyclooxygenase-2); Rabbit, polyclonal; 1:1000 dilution; secondary antibody was anti-rabbit-AP (Sigma, A3937, 1:1000).

iNOS staining was performed using a polyclonal anti-iNOS, Alexis, 1:1000). Blots were washed in TBS/1.0 % Tween and incubated with the appropriate secondary antibody-horseradish peroxidase conjugate (anti-rabbit IgG, Sigma, 1:2000).

1.4.2 HDAC-1 activity assay

For measurements of HDAC activities, the quantitative test kit for NAD-dependent histone deacetylase activity CycLex® Sir2 Assay kit (Cat# CY-1151) was used according to instructions of manufacture (CycLex Co., Ltd. 1063-103 Ohara, Tera-Sawaoka Ina, Nagano 396-0002 Japan). All substances tested in the SIR assay have to be cross-checked for their influence on the lysyl-endopeptidase. For this control an already deacetylated substrate peptide is used in order to measure directly lysyl-endopeptidase activity.

1.4.3 LPS experiments

LPS challenge of 3T3 fibroblasts, A 549 cells, V56 embryonic stem cells and neurally differentiated V56 embryonic stem cells was equally performed by exposing cells to 100 ng/ml lipopolysaccharide (LPS, E.coli 0111:B4 LPS from Sigma) for 60 min in the presence or absence of nelfinavir or saquinavir and related compounds respectively. Cell pellets were further investigated by Western blot staining with anti cox-2 and anti iNOS antibodies of 1D polyacrylamide gels.

1.4.4 ADP-ribosylated proteins

Poly-ADP ribosylated proteins were quantified from appropriate cellular protein extracts by Western blots using as primary antibody: Anti-poly(ADP-ribose); Antigen: mouse; Biomol, Cat Nr SA-216, Lot Nr. : P7482; and as secondary antibody: Anti mouse, AP; Sigma A 9316, Lot: 31K 9205; both antibodies were used at 1:2000 dilution.

1.4.5 AIF kinetics, Western blots

For quantification of release of apoptosis inducing factor (AIF) from mitochondria after ischemic/ excitotoxic or β-amyloid related insults the cellular material was fractionated into cytosolic, nuclear and mitochondrial fractions according to Arnoult et al. (Damien Arnoult, Philippe Parone, Jean-Claude Martinou, Bruno Antonsson, Jérôme Estaquier, and Jean Claude Ameisen Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome *c* release in response to several proapoptotic stimuli Journal of Cell Biology, Volume 159, Number 6, December 23, 2002 923–929).

Staining of related Western blots was performed using as primary antibody: anti AIF (apoptosis inducing factor); Antigen: goat polyclonal; Sc-9416 from

Santa Cruz; at a dilution of 1:2000; and as secondary antibody anti-goat IgG, whole molecule; developed in rabbit, A 4174 Sigma , peroxidase-coupled at a dilution of 1:1000.

2. Results

2.1 Neuroprotection in vitro

Neuroprotective effect of Nelfinavir/Saquinavir in chemical ischemia, excitotoxic and β-amyloid-induced neuronal insult

Figure 5 shows the neuroprotective effect of nelfinavir and saquinavir in the functional model outlined in the methods section at concentrations of each 1 µM.

Whereas control cells here had a survival rate of 5.9 ± 3.3%, nelfinavir (1µM)-treated cells had a survival rate of 91.0 ± 3.8%, and saquinavir (1µM)-treated cells had a survival rate of 59.5 % ± 5.5%, with EC50 of approx. 100 nM (nelfinavir) and 950 nM (saquinavir) under ischemic conditions.

As a summary of neuroprotective effects of nelfinavir/saquinavir in three different insults the following was observed: After induction of chemical ischemia as described we found EC 50 values of about 100 and 950 nM, respectively. After induction of excitotoxic cell death by 100 µM NMDA (or 100 µM HCA as in Sommer S. et al., supra, (2004)) we found EC 50 values of 1000 nM in both cases and after induction of neuronal death by 10 µM β-amyloid1-40 (Bachem, Germany), we found an EC 50 value of 500 nM in both cases; All three insults induce an initial calcium overload, which obviously initiates proapoptotic and proinflammatory events, leading eventually to neuronal dysfunction and cell death. A decrease of apoptotic and inflammatory markers PARP-1, AIF, cox-2 and iNOS during neuroprotective treatment with nelfinavir/saquinavir and acetylnelfinavir/saquinavir in the in vitro model was found.

2.2 Chemical Proteomics

2.2.1 Identification of VDAC-1, REA and prohibitin as targets of nelfinavir/saquinavir

We then proceeded to synthesize reactive nelfinavir/saquinavir derivatives as shown above. Nelfinavir was used as a starting structure for the synthesis of the affinity reagent, which was used to bind the targets from fractionations of crude cell extracts of D3 embryonic stem cells. Subsequently the affinity purified material was analysed by 1D PAGE (Fig. 6) and mass spectrometry. MALDI-TOF analysis of the silver stained gels indicated the presence of VDAC-1 in specifically enriched fractions.

2.2.2 Electrophysiological VDAC-1 Assay

cDNAs for Homo sapiens prohibitin (PHB) and Homo sapiens voltage-dependent anion channel 1 (VDAC-1) were purchased from Origene Technologies Inc. USA. VDAC-1 was expressed in Xenopus laevis oocytes, alone and together with prohibitin. Electrophysiological measurements show that Nelfinavir and Saquinavir are inhibitors of VDAC-1 and that prohibitin is modulating this inhibitory effect.

2.2.3 REA assay

The possibility that REA interacts with histone deacetylases (HDAC) prompted us to test nelfinavir/saquinavir and derivatives in a quantitative test kit for NAD-dependent histone deacetylase activity (CycLex Assay kit): The substances appear to be inhibitors of HDAC activity.

2.3 General cytoprotective effects towards an inflammatory insult (LPS exposure)

We stimulated 3T3 fibroblasts, A549 cells, undifferentiated V56 embryonic stem cells and neurally differentiated V56 embryonic stem cells with 100 ng/ml lipopolysaccharide (E.coli 0111:B4 LPS from Sigma) for 60 min. As an inflammatory marker we again quantified expression of cox-2 and iNOS by appropriate antibody staining of Western blots of 1D PA gels. The results show that nelfinavir/saquinavir and related substances i) protect cells from LPS-induced death, and ii) that this protective effect is accompanied by a decreased expression of inducible inflammatory markers iNOS and cox-2. Cell survival was assessed by Trypan Blue staining.

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Next to the direct effect on VDAC-1, the substances appear to bring about their effects via transient membrane domains, cholesterol-rich lipid rafts, which are thought to be an important in a variety of related signalling pathways, in particular in the context of HIV infection (Cuschieri J. Implications of lipid raft disintegration: enhanced anti-inflammatory macrophage phenotype. Surgery. 2004 Aug;136(2):169-75.; Chu CL, Buczek-Thomas JA, Nugent MA. Heparan sulphate proteoglycans modulate fibroblast growth factor-2 binding through a lipid raft-mediated mechanism. Biochem J. 2004 Apr 15;379(Pt 2):331-41; Argyris EG, Acheampong E, Nunnari G, Mukhtar M, Williams KJ, Pomerantz RJ. Human immunodeficiency virus type 1 enters primary human brain microvascular endothelial cells by a mechanism involving cell surface proteoglycans independent of lipid rafts. J Virol. 2003 Nov;77(22):12140-51; Nagy P, Vereb G, Sebestyen Z, Horvath G, Lockett SJ, Damjanovich S, Park JW, Jovin TM, Szollosi J. Lipid rafts and the local density of ErbB proteins influence the biological role of homo- and heteroassociations of ErbB2. J Cell Sci. 2002 Nov 15;115(Pt 22):4251-62).

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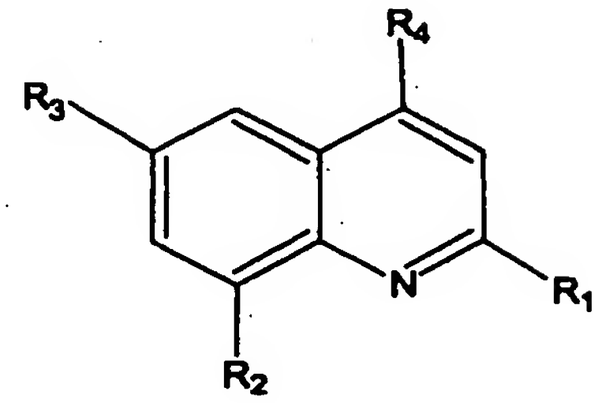
Claims

1. Use of a compound of the formula



(1)

wherein

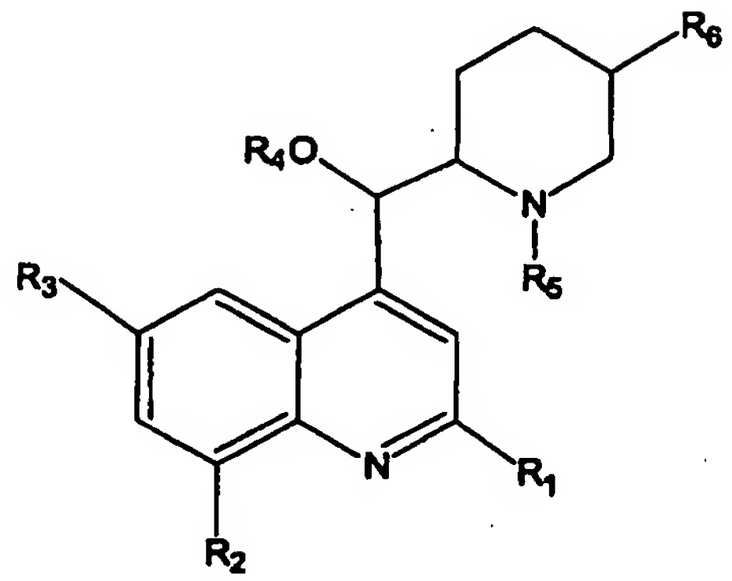
R_1, R_2 and R_4 are independently from each other hydrogen, C_1-C_8-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, wherein the alkyl or cycloalkyl group is optionally substituted with a five- or six-membered ring optionally containing at least one heteroatom selected from N, S and O, and wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl, C_1-C_4-(halo)alkoxy, amino, C_1-C_4-alkylamino, di(C_1-C_4-alkyl)amino or Z, wherein Z is a C_1-C_8-(halo)alkyl group ω-substituted with a group $-NR_5R_6$, wherein R_5 and R_6 are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_5 and R_6 together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl and C_1-C_4(halo)alkoxy and

R_3 is hydrogen, C_1-C_8-(halo)alkyl, C_3-C_8-(halo)cycloalkyl, or $-NR_7R_8$ wherein R_7 and R_8 are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_7 and R_8 together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-

(halo)alkyl and C_1-C_4-(halo)alkoxy,

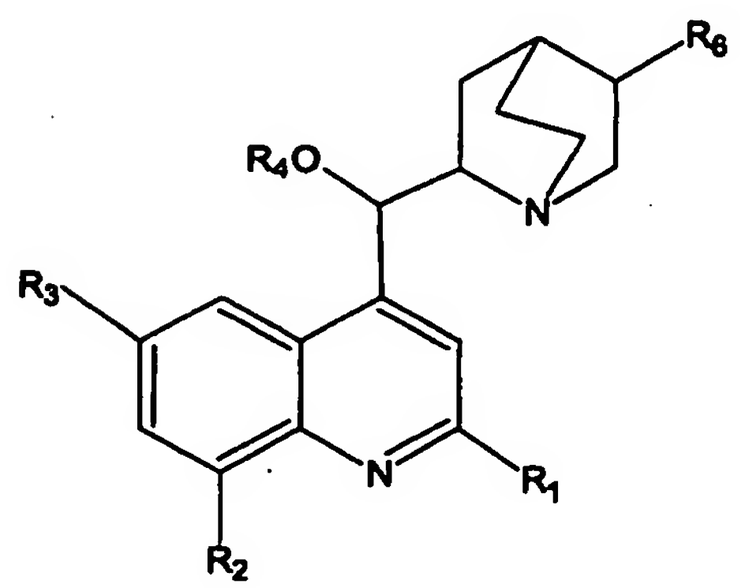
or

a compound of the formulae



(2)

or



(3)

wherein

R_1 is hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, wherein the alkyl or cycloalkyl group is optionally substituted with a five- or six-membered ring optionally containing at least one heteroatom selected from N, S and O, and wherein the ring is optionally mono- or poly-substituted with halo, C_1-C_4-(halo)alkyl, C_1-C_4-(halo)alkoxy, amino, C_1-C_4-alkylamino, di(C_1-C_4-alkyl)amino or Z, wherein Z is a C_1-C_6-(halo)alkyl group ω-substituted with a group

$-NR_7R_8$, wherein R_7 and R_8 are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_7 and R_8 together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl and C_1-C_4-(halo)alkoxy,

R_2 is hydrogen, halogen, C_1-C_8-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, $-NR_9R_{10}$, wherein R_9 and R_{10} are independently from each other hydrogen, C_1-C_8-alkyl, or CO-C_1-C_8-alkyl or wherein R_9 and R_{10} together form a five- or six-membered ring optionally containing at least one further heteroatom selected from N, S and O, wherein the ring is optionally monosubstituted up to polysubstituted with halo, C_1-C_4-(halo)alkyl and C_1-C_4-(halo) alkoxy,

R_3 is hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, halogen, OR_{11}, wherein R_{11} is C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl,

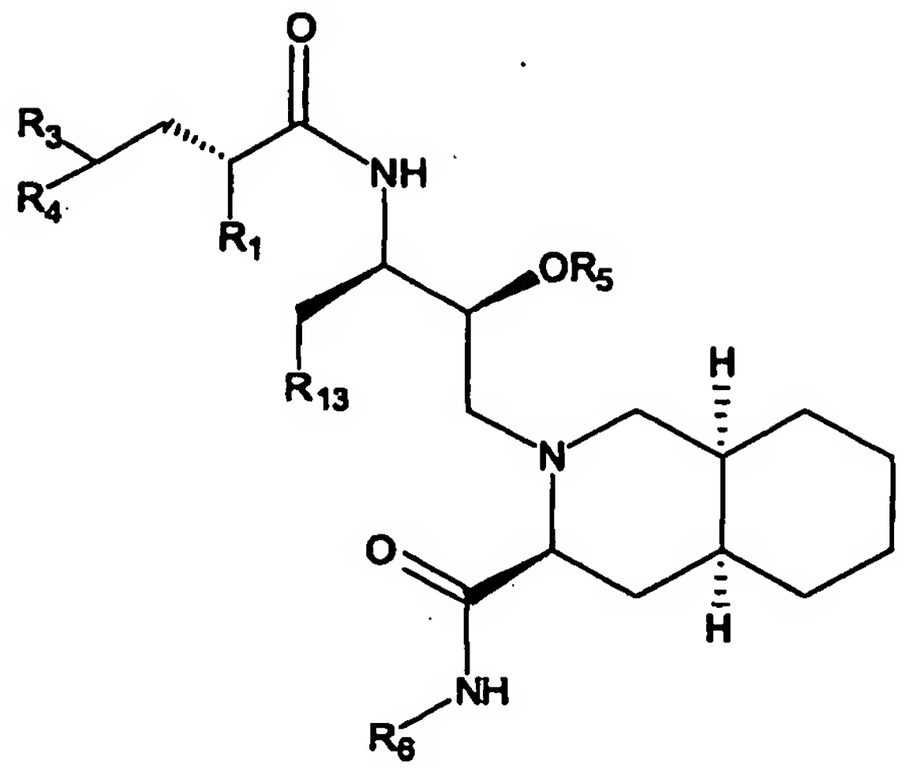
R_4 is hydrogen, C_1-C_6-(halo)alkyl, or C_3-C_8-(halo)cycloalkyl, CO-C_1-C_8-alkyl,

R_5 is hydrogen, C_1-C_6-(halo)alkyl, C_3-C_8-(halo)cycloalkyl or CO-C_1-C_8-alkyl and

R_6 is hydrogen, C_1-C_6-(halo)alkyl, C_3-C_8-(halo)cycloalkyl or C_2-C_6-alkylnyl,

or

a compound of the formula

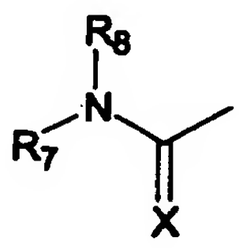


(4)

wherein

R_1 is hydrogen, hydroxy or NHR_2,

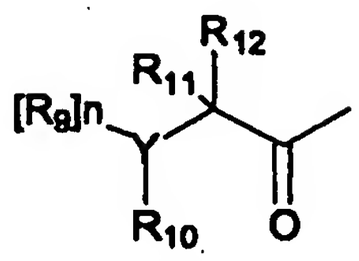
R_2 is hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heterocyclylalkyl, cycloalkyl, alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, arylalkylcarbonyl, heterocyclylalkylcarbonyl, alkoxycarbonyl, arylalkyl oxycarbonyl, heterocyclylalkoxycarbonyl, aryl, heterocyclyl, sulfonyl, alkylsulfonyl, arylsulfonyl, heterocyclylsulfonyl or a group of the formula



wherein X is O or S and

R_7 and R_8 are independently from each other hydrogen, alkyl, aryl, heterocyclyl, arylalkyl, heterocyclyl alkyl or

R_7 and R_8 together with the nitrogen atom to which they are attached form a saturated ring optionally containing a further heteroatom or a group



wherein R_{10} is hydrogen, alkyl, arylalkyl, heterocyclylalkyl, aryl, heterocyclyl when n=0 and Y is O or S or

R_{10} is hydrogen, alkyl, arylalkyl, heterocyclylalkyl, aryl, heterocyclyl when n=1, Y is N, R_9 is hydrogen or alkyl or

R_9 and R_{10} form together with the heteroatom to which they are attached a heterocyclic ring when n=0 and Y is N, O or S, and

R_{11} and R_{12} independently are hydrogen or alkyl or

R_{11} and R_{12} form together with the carbon atom to which they are attached a ring,

R_3, R_4 are independently from each other hydrogen, alkyl, carbamido, or

R_3, R_4 form together with the carbon atom to which they are attached a carbocyclic ring,

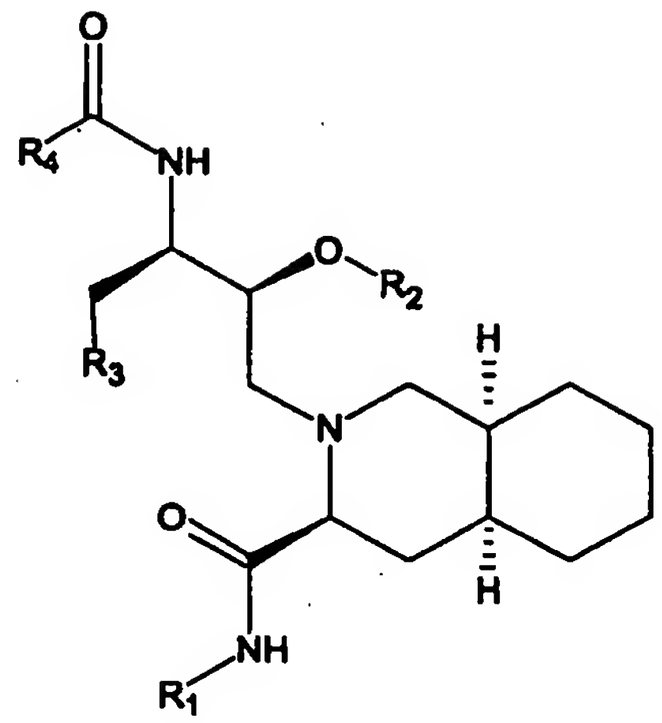
R_5 is hydrogen or the residue of an inorganic or an organic ester and

R_6 is alkyl, arylalkyl, heterocyclylalkyl, alkyloxyalkyl, hydroxyalkyl, amino alkyl, fluoroalkyl and

R_{13} is aryl, ω-alkylaryl, ω-alkylarylether or ω-alkylarylthioether,

or

a compound of the formula



(5)

wherein

R_1 is alkyl, arylalkyl, heterocyclylalkyl, alkoxyalkyl, hydroxyalkyl, alkylamino, aminoalkyl, fluoroalkyl,

R_2 is hydrogen or the residue of an inorganic or an organic ester,

R_3 is aryl, ω-alkylaryl, ω-alkylaryl ether or ω-alkylaryl thioether and

R_4 is aryl

or an optical isomer, a salt or derivative thereof for the manufacture of a cytoprotective medicament,

with the exemption of the use of mefloquine for the prevention or treatment of Alzheimers's disease or Parkinson's disease.

2. The use according to claim 1 for the manufacture of a medicament for the prevention or treatment of a disease associated with an inflammatory component.

3. The use according to claim 1 or 2 for the manufacture of a medicament for the prevention or treatment of a neurological or non-neurological inflammatory disease.

4. The use according to any of claims 1-3 for the manufacture of a

medicament for the prevention or treatment of disorders associated with apoptotic or neuroinflammatory, or generally inflammatory disorders which are caused by and/or accompanied by mitochondrial dysfunction.

5. The use according to any of claims 1-4 for the manufacture of a medicament for the prevention or treatment of a dysfunctional mitochondrial transition pore, and/or disorders which are caused by and/or accompanied by HDAC-dysfunction, particularly a dysfunctional decrease in HDAC activity.

6. The use according to any of claims 1-5 for the manufacture of a medicament for the prevention or treatment of neurodegenerative or neuroinflammatory diseases or pathological conditions, like M. Alzheimer, traumatic brain injury, M. Parkinson, amytrophic lateral sclerosis (ALS), stroke, migraine and multiple sclerosis.

7. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention or treatment of the common lung diseases associated with a significant inflammatory component such as severe sepsis, acute lung injury, acute respiratory distress syndrome, cystic fibrosis, asthma, allergic rhinitis, or COPD or lung cancer, as well as any other cancer type.

8. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention or treatment of cystic fibrosis, particularly in persons with impaired function of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel.

9. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention or treatment of ulcerative or other inflammatory conditions of the gastrointestinal system, particularly of persons with impaired function of

the cystic fibrosis transmembrane conductance regulator (CFTR) Cl channel.

10. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention or treatment of inflammatory processes involved in cancer prevention or progression.

11. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention or treatment of inflammatory processes involved in autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus, especially for the treatment of patients identified by genetic or other markers to be more likely to be especially amenable to such treatment.

12. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention and treatment of pain, particularly inflammatory or neurological pain.

13. Use of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 for the manufacture of a medicament for the prevention or treatment of inflammatory or inflammation-associated ocular disorders, particularly macula degeneration.

14. The use according to claim 13 for the manufacture of a medicament for for the prevention or treatment of moist or dry macula degeneration, glaucoma, e.g. acute, primary, secondary or low angle glaucoma, diabetic retinopathy, anterior or posterior optical neuropathy, retinitis pigmentosa, neuritis nervi optici, or central artery obstruction.

15. The use according to any of claims 1 to 14 wherein the compound is selected from acetyl mefloquine, chloroquine, quenine, primquine, ablaquine, amodiaquine and pharmaceutically acceptable salts or

derivatives thereof.

16. The use according to any of claims 1 to 14 wherein the compound is selected from nelfinavir, saquinavir, amprenavir, indinavir , lopinavir , ritonavir, atazanavir and pharmaceutically acceptable salts or derivatives thereof.

17. A method for the treatment or prevention of a disease associated with an inflammatory component which comprises administering to said subject a therapeutically effective amount of a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1,

with the exemption of a method for the prevention or treatment of Alzheimers's disease or Parkinson's disease which comprises administering mefloquine.

18. A method according to claim 17, wherein the disease is a neurological or non-neurological inflammatory disease.

19. A method according to claim 17, wherein the diseases are disorders associated with apoptotic or neuroinflammatory, or generally inflammatory disorders which are caused by and/or accompanied by mitochondrial dysfunction.

20. A method according to claim 17, wherein the diseases are a dysfunctional mitochondrial transition pore, and/or disorders which are caused by and/or accompanied by HDAC-dysfunction, particularly a dysfunctional decrease in HDAC activity.

21. A method according to claim 17, wherein the diseases are neurodegenerative or neuroinflammatory diseases or pathological conditions, like M. Alzheimer, traumatic brain injury, M. Parkinson, amytrophic lateral sclerosis (ALS), stroke, migraine and multiple

sclerosis.

22. A method according to claim 17, wherein the diseases are common lung diseases associated with a significant inflammatory component such as severe sepsis, acute lung injury, acute respiratory distress syndrome, cystic fibrosis, asthma, allergic rhinitis, or COPD or lung cancer, as well as any other cancer type.

23. A method according to claim 17, wherein the disease is cystic fibrosis, particularly in persons with impaired function of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel.

24. A method according to claim 17, wherein the diseases are ulcerative or other inflammatory conditions of the gastrointestinal system, particularly of persons with impaired function of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel.

25. A method according to claim 17, wherein the diseases are inflammatory processes involved in cancer prevention or progression.

26. A method according to claim 17, wherein the diseases are inflammatory processes involved in autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus, especially for the treatment of patients identified by genetic or other markers to be more likely to be especially amenable to such treatment.

27. A method according to claim 17, wherein the disease is pain, particularly inflammatory or neurological pain.

28. A method according to claim 17, wherein the diseases are inflammatory or inflammation-associated ocular disorders, particularly macula degeneration.

29. A method according to claim 17, wherein the diseases are moist or dry

macula degeneration, glaucoma, e.g. acute, primary, secondary or low angle glaucoma, diabetic retinopathy, anterior or posterior optical neuropathy, retinitis pigmentosa, neuritis nervi optici, or central artery obstruction.

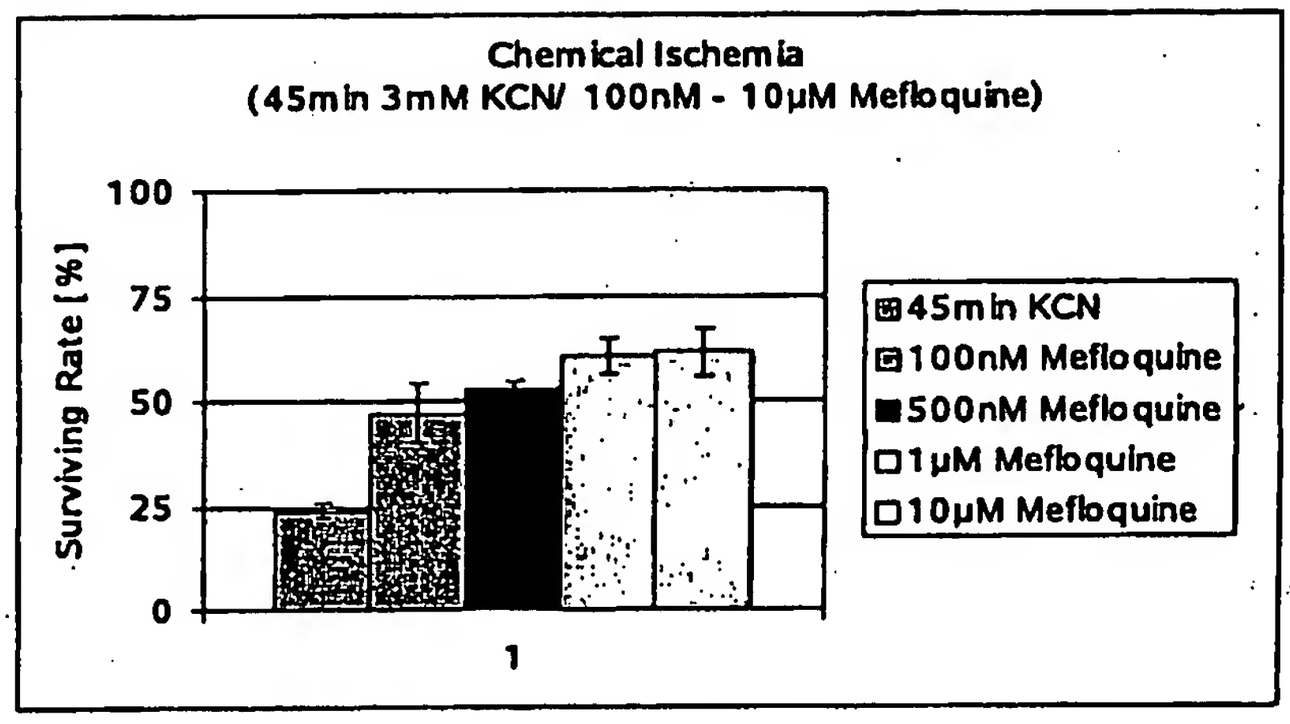
30. A method according to any of claims 17-29, which comprises administering to said subject an amount in the range from 1 to 2000 mg of a compound of formulae (1), (2), (3), (4) or (5) as defined in claim 1 per day.

31. Pharmaceutical composition comprising a compound of the formulae (1), (2), (3), (4) or (5) as defined in claim 1 and a pharmaceutically acceptable carrier, diluent and/or adjuvant.

32. A method according to any of claims 17-30, which method comprises administering to the subject a pharmaceutical composition as defined in claim 31 in the form of a tablet, capsule, solution, suspension, nasal spray, throat spray, gel or plaster.

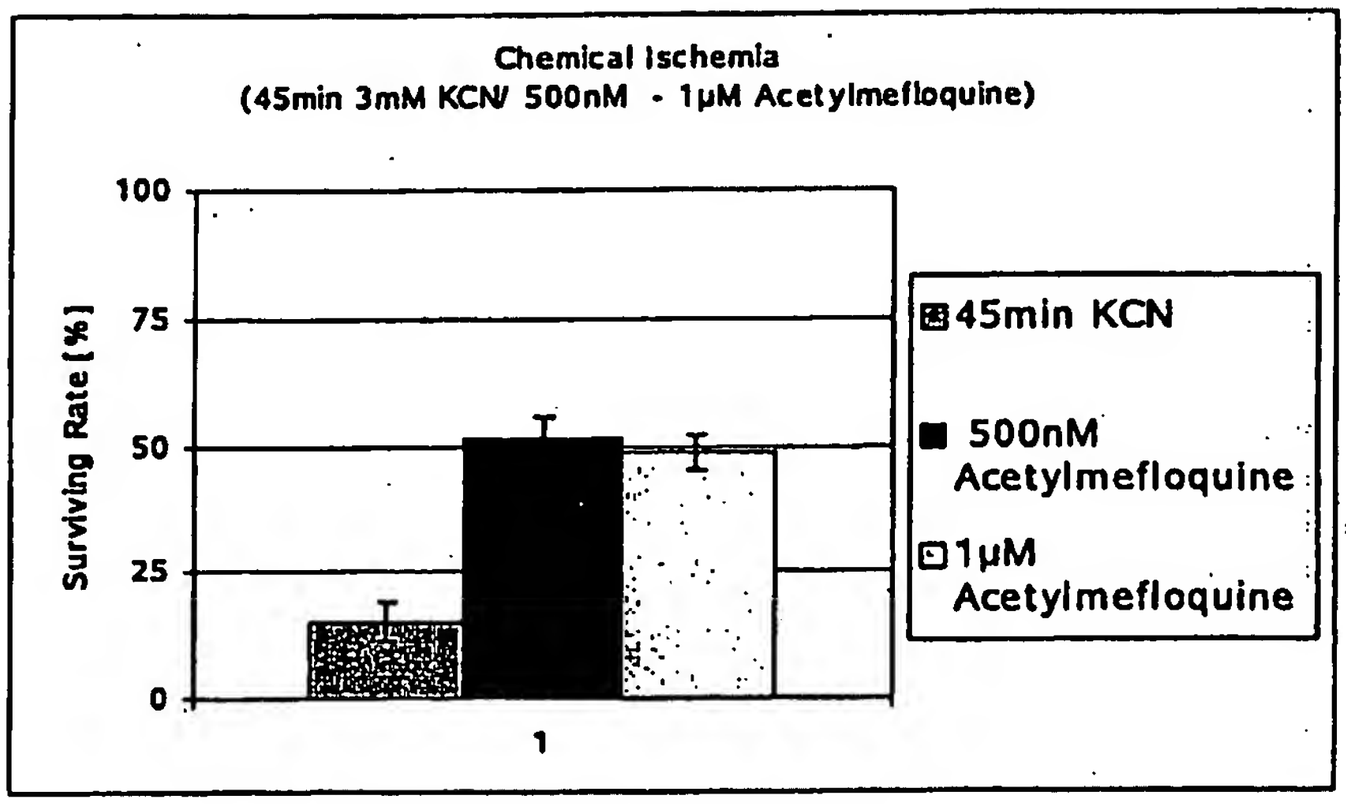
33. The use of a compound which is a MTP or REA inhibitor/modulator for the manufacture of a medicament for the prevention or treatment of airway diseases, preferably for the prevention or treatment of disorders as indicated above.

Figure 1



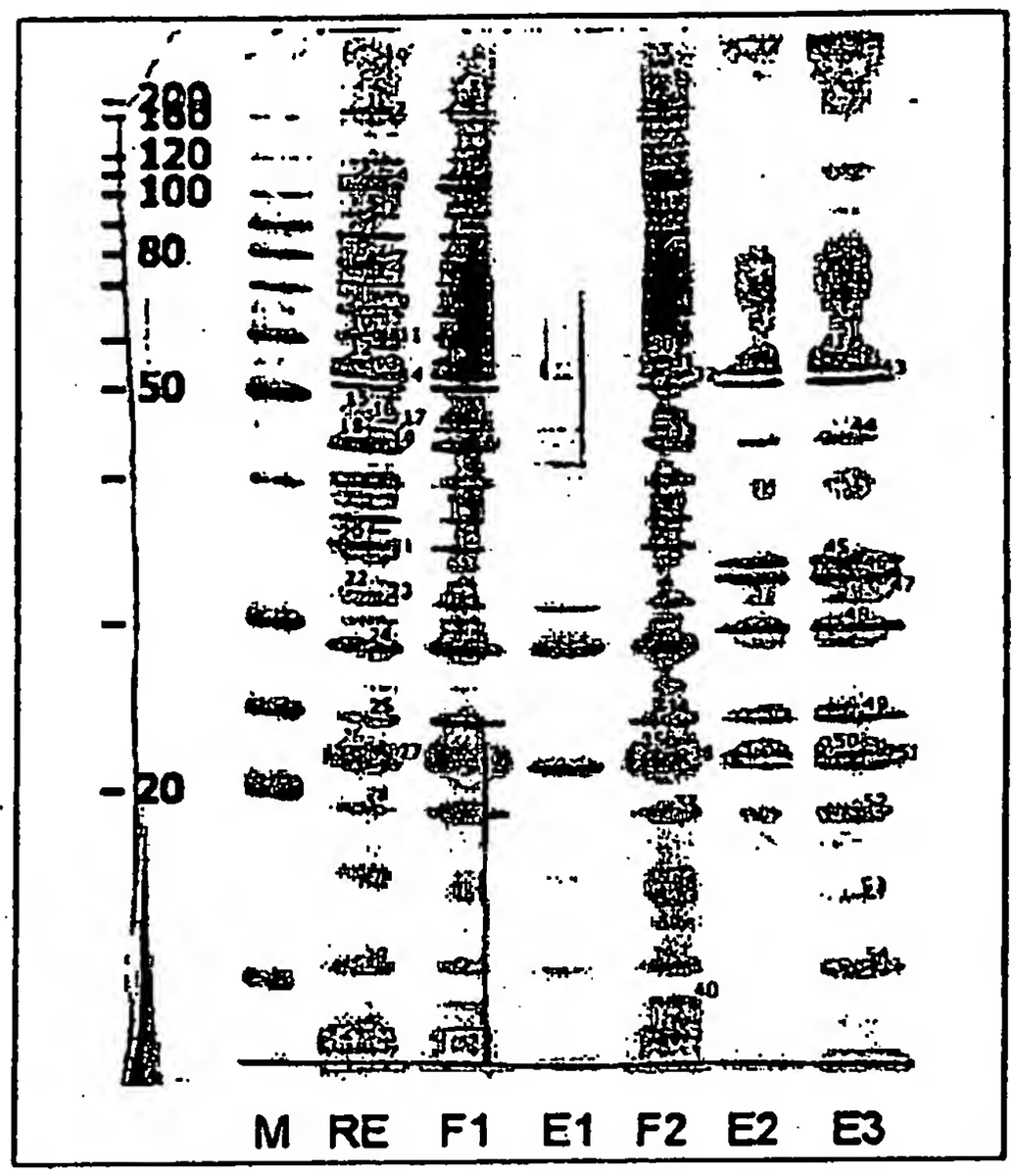
	# of functional neurons during 1.stimulation	# of functional neurons after chemical ischemia	Surviving rate [%]
Control	580	138	23.8 ± 1.8
100nM Mefloquine	51	24	47.1 ± 7.0
500nM Mefloquine	479	251	52.4 ± 2.3
1µM Mefloquine	127	77	60.6 ± 4.3
10µM Mefloquine	75	46	61.3 ± 5.6

Figure 2



	# of functional neurons during 1. stimulation	# of functional neurons after chemical ischemia	Survival rate [%]
Control	79	12	15.2 ± 4.0
500nM Acetylmefloquine	155	80	51.6 ± 4.0
1µM Acetylmefloquine	111	54	48.6 ± 3.6

Figure 3



Band #	MW_{app}	Mowse score	Accession #	pI	MW_{cal}	Name of protein
45	33000	149	gi|6005854	10.5	33276	Repressor of estrogen receptor activity [Homo sapiens]
		149	gi|34858436	10.4	33398	Similar to repressor of estrogen receptor activity; B-cell associated protein [Rattus norvegicus]
46	32000	257	gi|13786200	8.9	30737	Voltage-dependent anion channel 1 [Rattus norvegicus]
		253	gi|10720404	8.8	32331	Voltage-dependent anion-selective channel protein 1 (VDAC-1) (mVDAC1) (mVDAC5) (Outer mitochondrial membrane protein porin 1) (Plasmalemmal porin)
48	29000	169	gi|56206786	5.5	22958	Prohibitin (Mus musculus)

Figure 4

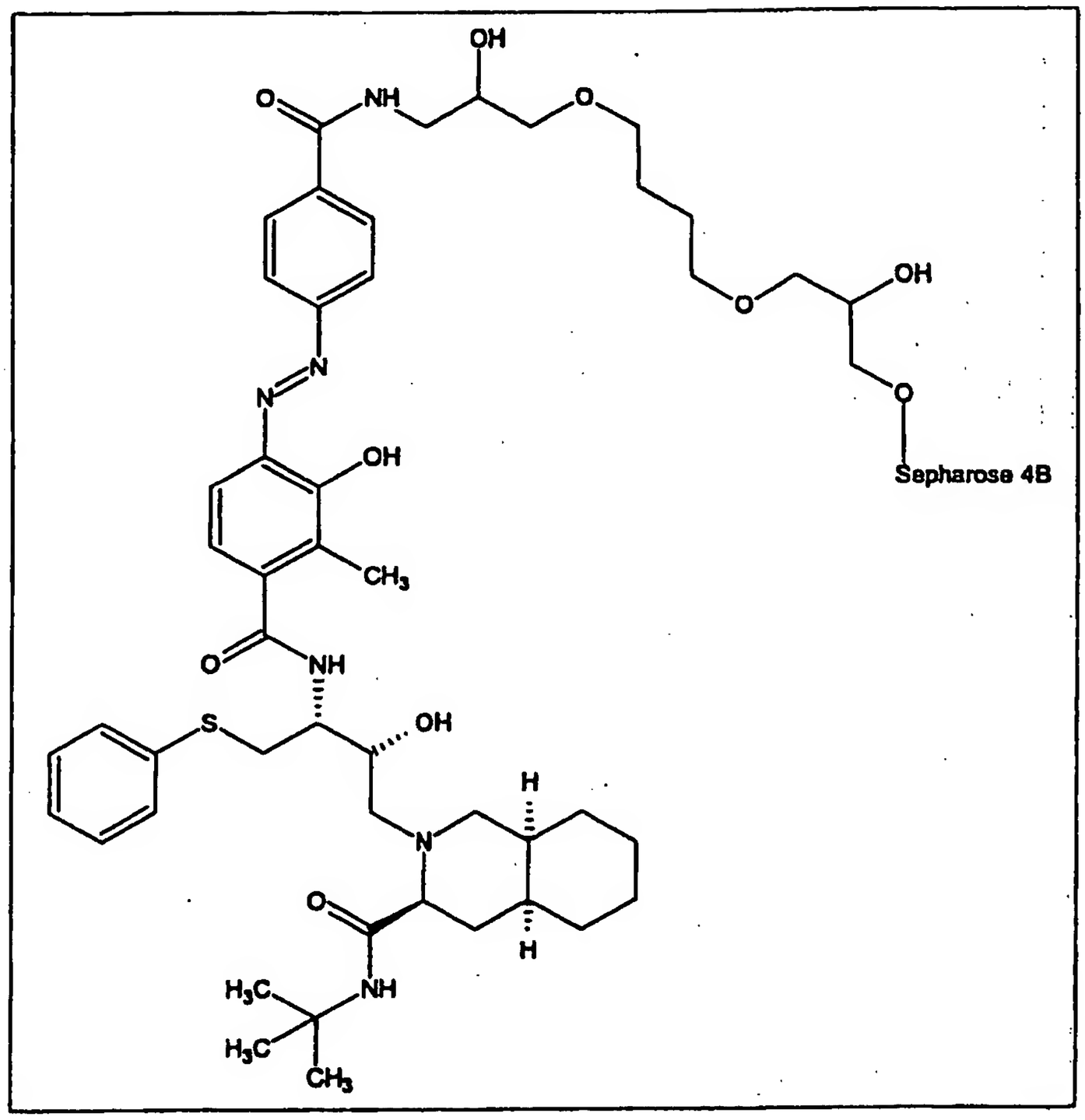
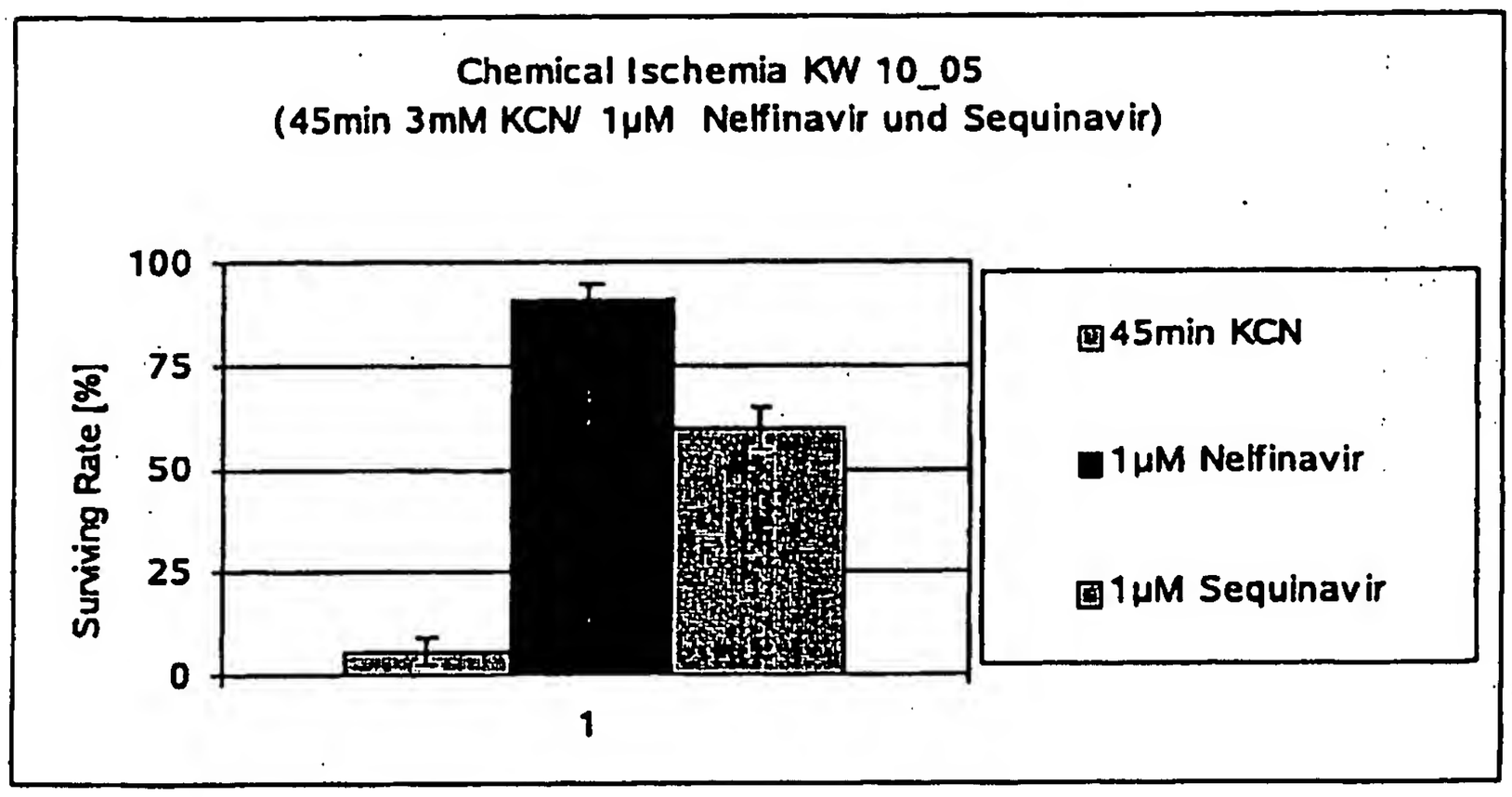
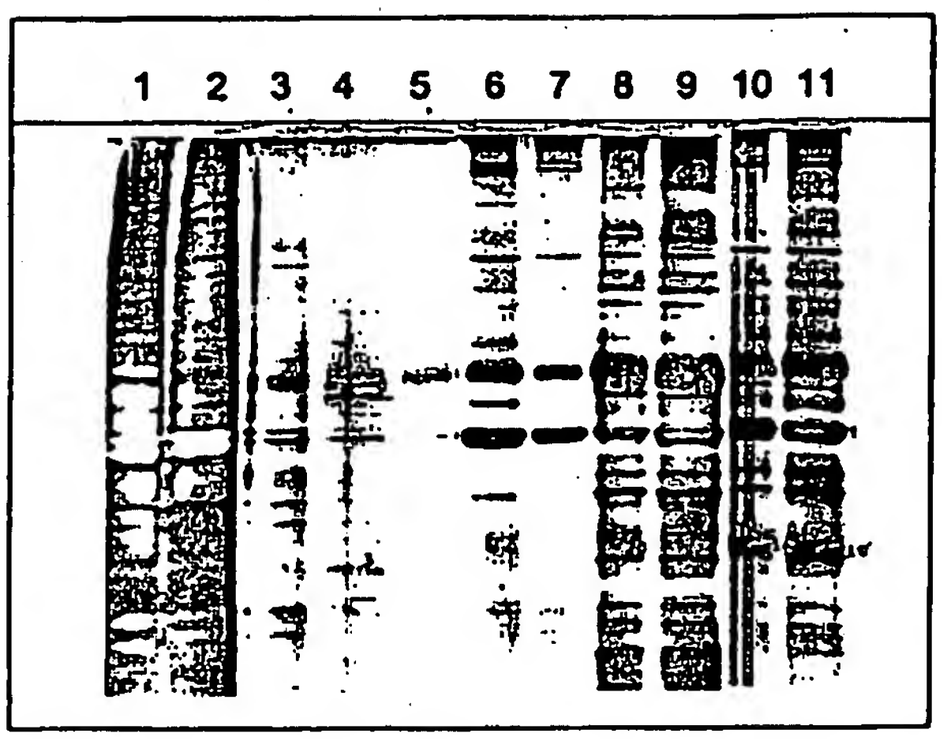


Figure 5



	Cells responding to control stimulus prior to ischemia	Surviving cells after chemical ischemia	Survival rate [%]
Control	51	3	5,9 ± 3,3
1µM Nelfinavir	56	51	91,0 ± 3,8
1µM Sequinavir	79	47	59,5 ± 5,5

Figure 6



14	B3	E15		34000	D357a	193	gi|13786200	8.9	30737	voltage-dependent anion channel 1 [Rattus norvegicus]
					D357a	191	gi|56585214		31409	Unknown (protein for IMAGE:7312189) [Rattus norvegicus]
15	B4	E16		33000	D357a	162	gi|13786200	8.9	30737	voltage-dependent anion channel 1 [Rattus norvegicus]
					D357a	161	gi|56585214		31409	Unknown (protein for IMAGE:7312189) [Rattus norvegicus]
16	B5	E17		32000	D357a	179	gi|56585214		31409	Unknown (protein for IMAGE:7312189) [Rattus norvegicus]
					D357a	167	gi|13786200	8.9	30737	voltage-dependent anion channel 1 [Rattus norvegicus]
17	B6	E18		33000	D357a	164	gi|13786200	8.9	30737	voltage-dependent anion channel 1 [Rattus norvegicus]
					D357a	162	gi|56585214		31409	Unknown (protein for IMAGE:7312189) [Rattus norvegicus]
18	B7	E19		33000	D357b	139	gi|56585214		31409	Unknown (protein for IMAGE:7312189) [Rattus norvegicus]
					D357b	128	gi|13786200	8.9	30737	voltage-dependent anion channel 1 [Rattus norvegicus]
19	B8	E20		32000	D357a	127	gi|56585214		31409	Unknown (protein for IMAGE:7312189) [Rattus norvegicus]
					D357b	116	gi|13786200	8.9	30737	voltage-dependent anion channel 1 [Rattus norvegicus]

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/003437

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4709 A61K31/4725 A61P17/06 A61P25/06 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 197 788 B1 (FLETCHER ALLAN ET AL) 6 March 2001 (2001-03-06) cited in the application column 5, lines 51-63 claims 1,9,21	1-6,15, 17-21, 30-33
X	WO 02/19994 A (ARAKIS LTD; SKEAD, BENJAMIN, MARK; BANNISTER, ROBIN, MARK; ROTHAUL, AL) 14 March 2002 (2002-03-14) claims 1,3,6	1-5,7, 9-11,15, 17-20, 22,24, 26,30-33

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 September 2006	20/09/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Veronese, Andrea

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/003437

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/216431 A1 (RAUT RAJEEV) 20 November 2003 (2003-11-20) paragraphs [0002] - [006,] paragraph [0022] paragraphs [0166], [169] claims 1,13,25-31	1-3,13, 14,28-32
X	US 2004/220221 A1 (BAKER HELEN FRANCES ET AL) 4 November 2004 (2004-11-04) paragraphs [0002], [0004], [0005], [0008] claims 1-9	1-5,7-9, 11,15, 17-20, 22,24, 26,30-32
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X	US 2005/020580 A1 (BADLEY ANDREW D ET AL) 27 January 2005 (2005-01-27) Se paragraph 37: sasquinaqvir, ritonavir, nelfinavir, ritonavir, indinavir paragraphs [0038] - [0044] claims	1-9,11, 13,14, 16-26, 28-33
X	WO 03/051361 A (CEDARS-SINAI MEDICAL CENTER) 26 June 2003 (2003-06-26) page 10, lines 1-8 page 11, lines 4-6 page 11, lines 12-21 page 12, lines 3-6 page 12, lines 7-19 examples claims 1,2,4,5,8-10,12,13,17,18	1-6,9, 11,12, 16-20, 24,26, 30-33

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/003437

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CRUIKSHANK SCOTT J ET AL: "Potent block of Cx36 and Cx50 gap junction channels by mefloquine." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 17 AUG 2004, vol. 101, no. 33, 17 August 2004 (2004-08-17), pages 12364-12369, XP002397598 ISSN: 0027-8424 the whole document	1-33
A	SHARMA AMITA ET AL: "Vi polysaccharide of Salmonella typhi targets the prohibitin family of molecules in intestinal epithelial cells and suppresses early inflammatory responses." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 14 DEC 2004, vol. 101, no. 50, 14 December 2004 (2004-12-14), pages 17492-17497, XP002397599 ISSN: 0027-8424 the whole document	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/003437

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 17-30, 32 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/003437

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